Neurodegenerative Disease and Treatments

P09-01

Apolipoprotein E gene polymorphism in Indian patients with Alzheimer's disease and vascular dementia

M. Tripathi, K. Luthra, A. B. Dey and R. Grover
Department of Neurology, All India Institute of Medical Sciences, New Delhi, India

The association of apolipoprotein E gene polymorphisms with Alzheimer's disease and vascular dementia has been reported in several populations including one from a rural community in North India. However, the association of Apo E polymorphism with VaD is yet to be established in this population.

Material and methods: In a case–control study involving 54 cases of dementia (AD-29 and VaD-25) and 76 age matched healthy controls blood was analysed for the Apoe polymorphism.

Results: The frequency of e4 allele was significantly higher among cases of AD and VaD compared with controls ($p < 0.001$). The e3e3 ($p < 0.05$) and e3d3 ($p < 0.001$) genotypes were found to be protective. The odds of developing AD or VaD were at 4.4 and 3.7 times higher respectively in presence of even a single even e4 allele.

Results: Our results suggests that the increased risk of developing AD or VaD is similar among Asian Indians in presence of apo e4 compared with Caucasian population.

Keywords: aging, Alzheimer's disease, aPOE, apolipoproteins, dementia.

P09-02

Amyloid beta peptides and apoE4 decrease phosphoinositides kinases activity in rat brain synaptic plasma membranes

A. Zambrzycka and J. B. Strosznajder
Department of Cellular Signaling Medical Research Centre, Warsaw, Poland

Phosphatidylinositol 4-kinase (PI4K), phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositolmonophosphate 5-kinase (PIP5K) that synthesize polyphosphoinositides (PI, PIP, PIP_2) are involved in intracellular signal transduction, cytoskeletal function and neuronal survival. It was indicated that PI4K and PI3K activity is decreased in the brain of patients with Alzheimer's disease (AD). However, the mechanism of inhibition is still unclear. As amyloid beta peptides (Aβ) in aggregated form are neurotoxic and apolipoprotein E4 (apoE4) accelerates their aggregation, we examined whether Aβ 1-42 and apoE4 may be responsible for the inhibition of phosphoinositides kinases activity. The aim of study was to investigate the effect of Aβ and apoE4 on these enzymes activity in cortical and hippocampal synaptic plasma membranes (SPM) with endogenous and exogenous inositolphospholipids and (γ32P)ATP. The results indicated that Aβ is responsible for the lower phosphorylation of endogenous PIP to PIP_2 in the brain cortex and hippocampus. This peptide and apoE4 decreased PI4K activity in the brain cortex by about 20 and 40% respectively. Preliminary data with wortmannin suggested that Aβ and apoE4 affected also PI3K. Moreover, apoE4 decreased PIP5K activity by 20% and Aβ has no effect on this enzyme. Arachidonic acid (AA) that is liberated by Aβ decreased phosphoinositides kinases activity. In conclusion, these studies suggest that inhibition of phosphoinositides kinases activity by Aβ and apoE4 might by responsible for the alteration of cytoskeleton dynamic and phosphoinositides signaling.

Keywords: Alzheimer's disease, amyloid, apolipoproteins, brain, phospholipids.

P09-03

Haloperidol stabilizes calcium-imbalance in Alzheimer's disease

A. Palotas, J. Kalman, M. Palotas, Z. Janka and B. Penke
Department of Medical Chemistry, University of Szeged, Szeged, Hungary

Deposition of beta-amyloid peptide is the diagnostic neuropathologic feature of Alzheimer's disease. One of the underlying pathomechanisms of beta-amyloid-induced cellular toxicity, based on the channel hypothesis, is the disruption of the intracellular calcium homeostasis of neurons, fibroblasts and lymphocytes. Piling evidence demonstrates a low frequency of Alzheimer's disease in patients with schizophrenia, and it has been proposed that antipsychotic medications, such as haloperidol, may be responsible. Haloperidol has been recently reported to be a calcium and calmodulin antagonist. Therefore we evaluated haloperidol for its effects on beta-amyloid-induced calcium-imbalance on readily available tissues, such as fibroblasts. Basal intracellular calcium-level was measured in Fura-2AM-loaded human fibroblasts by dual wavelength spectrofluorimetry. Alzheimer cells exhibited lower calcium-level when compared with the control cultures. Exposure of fibroblasts to beta-amyloid peptide resulted in increased calcium concentration of the control cells, but not of Alzheimer fibroblasts. Preincubation of control cultures with haloperidol blocked the beta-amyloid-induced elevation of calcium. This finding indicates that haloperidol efficiently attenuates ionic imbalance and suggests that it may serve as a potential agent in alleviating neurotoxic effects of beta-amyloid peptide. Also, haloperidol may be a useful lead in the development of an effective Alzheimer therapeutic agent.

Keywords: Alzheimer's disease, amyloid, calcium, haloperidol.

P09-04

Prion protein ubiquitination and proteasomal dysfunction in scrapie infection

B. S. Wong,* M. Whiteman,† J. Sassoon,‡ S. C. Kang,* R. Li,* T. Pan,* M. A. Smith,* G. Perry,* D. R. Brown,‡ T. Wisniewski§ and M. S. Sy*
**Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA; †Department of Biochemistry, National University of Singapore, Singapore; ‡Department of Biology and Biochemistry, Bath University, Bath, UK; §Department of Neurology and Pathology, New York University, New York, USA*

Although the key event in the pathology of prion diseases is believed to involve the conversion of cellular prion protein (PrPC) to the partial protease-resistant scrapie species termed PrPSc, the contributing factors to neurodegeneration in scrapie-infected animal are poorly understood. Accumulation of PrPSc in infected brains can overwhelm the ubiquitin-proteasome systems, which are important in maintaining cellular homeostasis. We found that in mouse brains infected with the ME7 scrapie strain, there is an increase in DNA breakage and a reduction in two endopeptidase activities associated with proteasome function. In addition, the decline of proteasome activity was accompanied by an elevation in the levels of total ubiquitin adducts. By using a capture-ELISA, we found that the PrP species from infected brains are ubiquitinated. These results suggest that increased DNA damage, impaired proteasome function and ubiquitination of the PrP species may play a pivotal role in the pathogenesis of prion diseases. Also, the ability to detect ubiquitinated PrP species in affected brains may serve as a potential marker for prion disease.

Keywords: neurodegeneration, oxidative stress, prion, scrapie, ubiquitin.

P09-05

Cyclohexylbisphenol inhibits oxidative stress in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) mouse model of Parkinson's

M. Chalimoniuk,* Y. Liu,† D. Kopczuk* and J. Strosznajder*
*Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; †The Salk Institute for Biological Studies, La Jolla, CA, USA

The mechanism of neuronal degeneration in Parkinson's Disease (PD) is up till now unknown. The previous studies suggest that the free radicals are involved in neuronal death in substantia nigra. Excessive synthesis of NO and cGMP altar physiological and biochemical processes in cells. The aim of our studies was to investigate effect of cyclohexylbisphenol (CHBP) on oxidative stress and cGMP level in MPTP animal's PD. Mice C57/BL received i.p. three injection of MPTP in saline at 3-h intervals in total dose of 40 mg/kg b.w. Control mice were injected saline only. After last injection of MPTP the same group of animals received one injection of CHBP i.p. in a dose of 40 mg/kg b.w. Mice were killed after 3, 7 and 14 days. The striatum, midbrain, hippocampus and brain cortex were isolated. Free radicals were assayed using fluorescence method (DCF), lipid peroxidation was evaluated by determination of thiobarbituric acid (TBARS). Moreover, cGMP and glutathione level were estimated using ELISA and spectrophotometric methods. Our results indicated that MPTP induced increase of oxidative stress (DCF, TBARS), cGMP level and depletion of glutathione in striatum and midbrain 3, 7 and 14 days after injection. CHBP, which express to have a strong antioxidant properties *in vitro*, also inhibited free radicals formation, lipid peroxidation and protected glutathione level against depletion in MPTP animal_fs PD. CHBP had no effect on MPTP induced elevation of cGMP in striatum and midbrain. These results suggest that CHBP can be useful in treatment of PD.

Keywords: anioxidant, cGMP, Parkinson's diease, superoxide dismutases.
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P09-06

Challenges and excitements in understanding of abeta peptides and aluminium induced helical transitions in supercoiled DNA and POL

S. Anitha and K. S. Jagannatha Rao
Central Food Technological Research Institute, Mysore, India

We have first time evidenced a BàZ conformational change in Alzheimer's brain genomic DNA in hippocampus. This finding lead to a hypothesis that Alzheimer's disease (AD) etiological factors like Aluminium (Al), amyloid beta peptide, Tau etc., might play role in modulating DNA topology (1). We further observed that Abeta and Al are localized in the nuclei in AD brain. This data gave impetus for the present study of Al and Abeta interaction with DNA. Spectroscopic studies have revealed that Abeta (1–42) could induce B à Psi conformational change in Supercoiled DNA (scDNA), Abeta (1–16) caused an altered B-form, while Al induced a complex B-C-A mixed conformation. Al induced Z-DNA in poly (GC) while showed strong binding to poly (AT). Abeta peptides (1–16, 1–28 & 1–40) induced A-DNA conformation in poly (AT), with no helicity change in poly (GC). Ethidium bromide binding and agarose gel studies revealed that Al linearized the sc DNA while Abeta (1–42) and Abeta (1–16) caused partial linearization and also showed differential sensitivity to Chloroquine towards topoisomers separation. This is the first report to show that Abeta and Al modulate helicity in scDNA, poly (AT) and poly (GC). This information may throw a new light in understanding molecular biology of Alzheimer's disease.

Keywords: Abeta, aluminium, Alzheimer's Disease, DNA, Z-DNA.

References

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P09-07

Aluminium amino acid complexes interaction with supercoiled DNA: a new evidence on DNA helicity change in relevance to Alzheimer's

D. Bharathi and K. S. Jagannatha Rao
Central Food Technological Research Institute, Mysore, India

Alzheimer's disesase (AD) is a complex neurological disorder with multiple etiology. In AD brain, racemized forms of amino acids in particular, D-Aspartate and D-Glutamate are relatively in large proportions in amyloid plaques and neuro-fibrillary tangles. Reports from our lab revealed that Aluminium (Al), an etiological factor of AD, favors stereoinvertion from L to D enantiomer in aged rabbit brain. Al,as Al-aminoacid complexes were able to cross the blood–brain barrier and deposit in the brain. Further, the observation that Al and Abeta(rich in D-Asp and D-Glu) are localized in the nuclei, provides an insight for the present study of Al-amino acid complexes interaction with DNA. Thereby L and D forms of Al-Aspartate and Al-Glutamate interaction with DNA could contribute to DNA conformational change. Circular Dichroism studies of Al-D-Asp interaction with supercoiled DNA revealed B to C DNA conformational change, while Al-L-Asp, Al-L-Glu & Al-D-Glu complexes showed strong binding to DNA. EDTA could reverse DNA conformation from C-B whereas desferoximine could not. Interstingly C-DNA induced by Al-D-Asp was treated with spermine elevated in AD, further induced an asymmetric condensation of 'limit C-motif' to a psi-DNA. The results are also supported by Gel studies, EtBr binding pattern. The spacial arrangement of molecules were understood by carrying out computer modeling studies. The differential DNA binding property of Al-amino acid complexes is dictated by the stereoisomerism and chirality of the complexes. This is the first study which provides a novel understanding on the role of Al-D-Asp modulating DNA conformations relevance to AD.

Keywords: Aluminium-D-Aspartate, Alzheimer's disease, C-DNA, psi-DNA, spermine.

P09-08

Modeling of trace elemental inter-relationships in serum samples of neuro-degenerative (Alzheimer's, Parkinson's diseases) and neu

P. Shanmugavelu, T. S. Sathyanarayana Rao, M. L. Hegde, M. S. Mushtaque, D. Bharathi, S. Anitha, R. B. Menon, R. V. Rao and K. S. Jagannatha Rao
Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India

The data on trace elements in different vital organs in normal and pathological conditions is very essential to establish a cause and effect relationship. Analysis of trace level elements were carried out using 'Inductively Coupled Plasma Atomic Emission Spectrometry' (ICPAES) in brain and CSF of Alzheimer's disease (AD), and serums of Parkinson's disease (PD) and Bipolar disorder (BPD). The results indicated a definite pattern of inter-elemental relationships. In moderately affected AD brains, monovalent elements were significantly reduced, while divalent elements were increased compared with normal brain. In severely affected AD brains, Al, S, Fe were predominantly present displacing other elements. It was found that Zn and Fe accumulate more in moderate, while Fe and Al deposition was more in severely affected AD. In severely affected AD brain regions, trivalent elements (Al and Fe) were predominantly present over monovalent and divalent elements compared with normal brain. In case of CSF samples, Al, Mg, Mn and Ca levels did not show any significant change between normal and AD CSF. However, K, P, and S were ($p < 0.0001$) decreased significantly, while Na level was ($p < 0.0001$) increased in AD CSF. Trace elements of serum in PD and BPD clearly showed a significant change for few elements in comparison with control. The observations are novel and significant. A comprehensive database was developed to inter-relate serum, CSF and brain on normal, PD, AD and BPD. Also the role of trace elemental homeostasis in neuro-degenerative and neuro-psychiatric disorders was hypothesized.

Keywords: inter-realtionships, modeling, neurodegenerative disorders, neuro-psychistry disorders, trace metals.

P09-09

Impairment of signal pathway for neuronal survival in hippocampus of Alzheimer's disease

S. Shuli, M. Yan and J. Zhijuan
Beijing Research Laboratory for Brain Aging, Xuanwu Hospital, Capital University of Medical Sciences, Beijing, China

The neuronal loss is one of AD neuropathological characteristic but the mechanism is still not well understand. In order to probe into the cause of neuronal loss, we observed the expression change of several proteins related to the signal transduction pathway for neuronal survival and apoptotic cascade for neuronal death in the AD (six cases) and non-AD (five cases) hippocampus. By application of immunohistochemistry and immunoprecipitation-Western blot, we found that Tunel positive cell in AD was one time more than in the non-AD control on basis of the neuron number. The expression of Akt/PKB, CREB (c-AMP response element binding protein), p-CREB (phosphorylated CREB) and Bcl-2 in AD were diminished. AIF (apoptosis-inducing factor) expression was increased. But Bax expression was not change. The ratio of Bcl-2 and AIF in AD was 0.47, but in non-AD was 0.99. It appears that AIF increase is very important. Our preliminary date suggest that neuronal survival signal pathway was impaired. It is possible to lead neuronal apoptotic cascade and neuronal loss.
Key words: Alzheimer's disease, hippocampus, neuronal survival, signal pathway.

P09-10

Aluminium amino acid complexes interaction with supercoiled DNA: a new evidence on DNA helicity change in relevance to Alzheimer's

D. Bharathi and K. S. Jagannatha Rao
Biochemistry and Nutrition Department, Central Food Technological Research Institute, Mysore, India

Alzheimer's disease (AD), a complex neurological disorder with multiple etiology. In AD brain, racemized forms of amino acids in particular, D-Asp and D-Glu are relatively high in Abeta neuro-fibrillary tangles. Reports from our lab revealed that Aluminium (Al), an etiological factor of AD, favors stereoinvertion from L to D enantiomer in aged rabbit brain. Al in the form of Al-amino acid complexes were able to cross the blood–brain barrier and deposit in the brain. Further, the observation that Al & Abeta(D-Asp) are localized in the chromatin region of the nuclei, provides an insight for the present study of Al-amino acid complexes interaction with DNA. Circular Dichroism studies of Al-D-Asp interaction with supercoiled DNA revealed B-C conformational change, while Al-L-Asp, Al-L-Glu & Al-D-Glu complexes showed strong binding to DNA. EDTA could reverse DNA conformation from C-B whereas desferoximine could not. Interestingly C-DNA induced by Al-D-Asp treated with spermine (elevated in AD) transformed the 'limit C-motif' into asymmetrically condensed chiral Psi-DNA. Gel studies indicated that Al amino acid complexes did not affect the supercoiling of DNA. Only Al-D-Asp-DNA complex showed sensitivity towards Chloroquine induced topoisomers separation. The differential DNA binding property of Al-amino acid complexes is dictated by the stereo's and chirality of the complexes. This is the first study which explores newer understanding on the role of Al-D-Asp modulating DNA conformation in the pathogenesis of AD.
Key words: Al-D-Asp, Alzheimer's disease, C-DNA, psi-DNA, spermine.

P09-11

Morphological and molecular tau pathology in the white matter of Alzheimer-type dementia

K. Ikeda, T. Arai, H. Akiyama and S. Iritani
Department of Psychogeriatrics, Tokyo Institute of Psychiatry; Department of Psychiatry, Tokyo Metropolitan Matsuzawa Hospital, Tokyo, Japan

White matter changes in ATD are a matter of argument. Secondary axonal degeneration and/or vascular change are assumed to their origin. We investigated whether there were primary tau pathology in the cerebral white matter using following methods. Tissues obtained from six patient of ATD at autopsy are fixed in 70%-ethanol in 150 mM NaCl or 4%-PFA in 0.1 M phosphate buffer. Sections were immunostained by 14 kinds of tau antibodies, which recognize various epitopes of tau. Immunoelectron microscopy was also performed. Ethanol fixed sections revealed massive tau-positive thread-like structures in the white matter, which scarcely observed in 4%-PFA fixed sections. Antibodies recognizing repeat-region (core part of tau) could not reveal these thread-like structures. On immunoelectron microscopy, these threads coincided with axons. Occasionally amorphous structures among fibrillar solid structures were immunolabelled. There were extensive axonal degeneration in the white matter of ATD. Immunoblotting revealed that the ratio of full-length tau is high in the white matter compared with gray matter. Deposited axonal tau in the white matter, differed from NFT or NT, seems not to undergo excessive processing.
Key words: Alzheimer-type dementia, pathology, tau, white matter.

P09-12

Enhanced expression of the P2X4 receptor in duchenne muscular dystrophy correlates with macrophage invasion

D. C. Gorecki,* D. Yeung,* R. Kharidia* and S. Brown†
**Institute of Biomedical and Biomolecular Sciences, School of Pharmacy and Biomedical Sciences, Portsmouth; †Dubowitz Neuromuscular Unit, Department of Paediatrics, Imperial College, Hammersmith Campus, London, UK*

Following molecular and immunohistochemical analysis of the purinergic P2X4 receptor in dystrophin-deficient muscle we have identified a distinct subpopulation of P2X4-positive cells infiltrating the dystrophic fibres. These cells were absent from normal muscle and rarely present in the dystrophic muscle taken before and after the onset of degeneration. We have identified these P2X4-positive cells as macrophages, demonstrating for the first time that human and mouse macrophages express P2X4 in addition to P2X7 receptors both *in vitro* and *in situ*, in specific tissues. Moreover, we demonstrated that the increase in the P2X4 expression is yet another feature of an inflammatory response identified in DNA arrays of dystrophic muscle. Immunohistochemical analysis failed to detect any expression of P2X4 protein in adult skeletal or cardiac muscle fibres, while myoblasts in culture expressed this receptor, as detected by RT-PCR and western blotting. This indicates developmental regulation of P2X4 expression in muscle. In the light of a significant role played by macrophages in the dystrophic process, the function of P2X receptors and their role in the Duchenne pathology as well as their potential role in therapeutic applications will be discussed.
Keywords: ATP, Duchenne muscular dystrophy, inflammation, macrophage, purinergic receptors.

P09-13

Dephosphorylation of microtubule-associated protein tau by protein phosphatase 5

C.-X. Gong,* F. Liu,* G. Wu,* S. Rossie,† J. Wegiel,* L. Li,* I. Grundke-Iqbal* and K. Iqbal*
**New York State Institute for Basic Research in Developmental Disabilities, Staten Island; †Purdue University, West Lafayette, USA*

Protein phosphatase (PP) 5 is a 58-kDa novel phosphoseryl/phosphothreonyl protein phosphatase. It is ubiquitously expressed in all mammalian tissues examined, with a high level in the brain, but little is known about its physiological substrates. We found that this phosphatase dephosphorylated recombinant tau phosphorylated with cAMP-dependent protein kinase and glycogen synthase kinase-3β, as well as abnormally hyperphosphorylated tau isolated from brains of patients with Alzheimer's disease. The specific activity of PP5 toward tau was higher than or comparable with those reported with other protein substrates examined to date. The PP5 activity toward tau was stimulated by arachidonic acid by 30–45-fold. Immunostaining demonstrated that PP5 was primarily cytoplasmic in PC12 cells and in neurons of postmortem human brain tissue. A small pool of PP5 associated with microtubules. Transfection of various PP5 plasmids into PC12 cells revealed that PP5 dephosphorylated tau in these cells. These results suggest that PP5 plays a role in the dephosphorylation of tau and thus can be involved in the molecular pathogenesis of Alzheimer's disease.
Keywords: Alzheimer's disease, dephosphorylation, protein phosphatase 5, tau protein.

P09-14

Impairments of cholinergic and noncholinergic neurotransmission in transgenic Tg2576 mouse brain with Alzheimer-like pathology

M. Klingner,* J. Apelt,* D. Sorger,† O. Sabri,† J. Steinbach,‡ M. Scheunemann‡ and R. Schliebs*
**Paul Flechsig Institute for Brain Research; †Department of Nuclearmedicine and ‡Institute of Interdisciplinary Isotope Research, University of Leipzig, Leipzig, Germany*

The cortical cholinergic deficits observed in brains from Alzheimer patients are highly correlated with the extent of neuropathological changes. To address the question whether beta-amyloid may contribute to these deficits, brain tissue from transgenic Tg2576 mice with Alzheimer plaque pathology at ages of 5 (still no significant plaque load) and 17 months (high cortical plaque burden) were examined for a number of cholinergic and noncholinergic markers. Transgenic mice with no significant plaque load demonstrated reduced hemicholinium (HC)-3 binding to choline uptake sites in anterior brain regions as compared with nontransgenic littermates, while in aged transgenic mice decreased HC-3 binding levels regionally correlated with high plaque load. The beta-amyloid-associated decrease in high-affinity choline uptake sites in some cortical brain regions was accompanied by an increase in vesicular acetylcholine transporter binding of vesamicol. Alpha1-, alpha2- and beta-adrenoceptor binding levels were hardly affected in aged transgenic mice. The data provide evidence of a modulatory role of beta-amyloid on cortical cholinergic neurotransmission but with differential actions on distinct cholinergic synaptic markers. The development of changes in cholinergic synaptic markers in transgenic Tg2576 mouse brain already before the onset of progressive plaque deposition provides *in vivo* evidence of a modulatory role of soluble beta-amyloid on cholinergic neurotransmission and may be referred to the deficits in learning and memory observed in these mice also before significant plaque load.
Keywords: acetylcholine, Alzheimer's disease, choline uptake, neurotransmission, receptor.

P09-15

Neuronal and glial expression of BACE1

S. Rossner, C. Lange-Dohna, G. von Geldern, U. Zeitschel, M. Hartlage-Rübsamen and V. Bigl
Paul Flechsig Institute for Brain Reseach, Leipzig, Germany

The beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE1) is a prerequisite for the generation of β-amyloid peptides, which give rise to β-amyloid deposits in the brains of Alzheimer's disease patients. It is believed that BACE1 is exclusively expressed by neurons. However, here we show that the BACE1 protein is also expressed by reactive astrocytes in proximity to β-amyloid plaques of APP transgenic mice and in animal models of chronic rather than acute gliosis. To identify elements which drive tissue- or cell type-specific BACE1 expression we cloned a 1.5 kb fragment of the rat BACE1 promoter and generated BACE1 promoter-luciferase reporter constructs. The basal activity of this promoter construct was highest in neuronal cell lines and in the pancreatic cell line AR42J, somewhat lower in rat primary neurons, astrocytic and microglial cultures, very low in hepatocytes and almost absent in fibroblasts and in the monocyte-macrophage cell line RAW264.7. Similar results were obtained in these cells by RT-PCR and by immunocytochemistry to detect BACE1 mRNA and protein, respectively. These data indicate that BACE1 is not a neuron-specific enzyme. We conclude that brain glial cells, in particular astrocytes, may contribute to increased generation of β-amyloid peptides and to accelerated formation of β-amyloid plaques in the course of Alzheimer's disease.
Keywords: aging, Alzheimer's disease, gene expression, glial cells, neurons.

P09-16

Expression of β-secretase BACE is differentially controlled through muscarinic acetylcholine receptor signalling

T. Zuchner and R. Schliebs
Paul Flechsig Institute for Brain Research, Leipzig, Germany

β-Amyloid peptides, derived by proteolytic cleavage from a much larger amyloid precursor protein (APP), play a major role in the pathogenesis of Alzheimer's disease by forming aggregated, fibrillary complexes that have been shown to be neurotoxic. Therefore, preventing β-amyloid formation by inhibition of APP-processing secretases is considered as potential strategy to treat Alzheimer's disease. Recently, the transmembrane aspartyl protease β-site APP-cleaving enzyme (BACE) has been identified as β-secretase. As cholinergic mechanisms have been shown to control APP processing, the present study intends to reveal whether the expression of BACE is particularly driven by muscarinic acetylcholine receptor (mAChR) using neuroblastoma cell line SH-SY5Y as a model. Stimulation of cells with the M1- and M3-selective mAChR agonist talsaclidine for 1 h resulted in a dose-dependent increase in BACE expression up to 2.5-fold over basal level, detectable 24 h following stimulation. Similar effects of BACE upregulation were observed, when protein kinase C (PKC) was directly activated by phorbol esters. In contrast, BACE expression is suppressed below the basal level by stimulating M2-mediated pathways by both selective M2-agonist binding or by direct activating of adenylate cyclase with forskolin. The data indicate that BACE expression is differentially controlled by mAChR signalling: agonist binding to M1/M3-mAChR and stimulation of PKC upregulates BACE expression, while BACE expression is downregulated through activation of M2/M4-mAChR and protein kinase A-mediated pathways. The data suggest that selective inhibition of brain M1/M3-mAChR signalling should be potential to suppress excessive β-amyloid formation in Alzheimer's disease.
Keywords: Alzheimer's disease, amyloid, BACE, muscarinic receptor, signal transduction.

P09-17

Interaction of interleukin-1β with muscarinic acetylcholine receptor-stimulated signaling cascade in cell culture

M. Gniezdzinska,* L. Kirazov,† A. Szutowicz‡ and R. Schliebs*
*Paul Flechsig Institute for Brain Research, University of Leipzig, Germany; †Institute of Experimental Morphology & Anthropology, Bulgarian Academy of Sciences, Sofia, Bulgaria; ‡Clinical Biochemistry, Department of Laboratory Medicine, Medical University of Gdansk, Poland

β-Amyloid plaque-mediated glial upregulation of the pro-inflammatory cytokine interleukin-1β (IL-1β) has been assumed to contribute to the impairments of cortical cholinergic neurotransmission observed in Alzheimer's disease. To test for this hypothesis, a murine cholinergic septal cell line SN56 was exposed to IL-1β followed by agonist stimulation of muscarinic acetylcholine receptors (mAChR) and detecting key molecules of both signaling cascades. The activities of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) of SN56 cells were dose-dependently increased following stimulation with carbachol, while stimulation of cells with the M1-mAChR-specific agonist talsaclidine did not affect cholinergic enzyme activities, suggesting that the carbachol-induced increases in cholinergic enzyme activities are mainly mediated through M2-mAChR signaling. Pre-exposure of SN56 cells to IL-1β (1ng/mL) for 1 h did not affect the carbachol-stimulated formation of inositol phosphates, but significantly induced the expression of ChAT mRNA and AChE activity while ChAT activity was not affected by IL-1β. Stimulation of IL-1β-pre-exposed cells with carbachol resulted also into upregulation of AChE activity and ChAT mRNA but to a lower extent as compared with incubations in the absence of carbachol, indicating interactive mechanisms between IL-1β and mAChR signaling cascades which may contribute to the cholinergic deficits in Alzheimer's disease.

Keywords: acetylcholine receptor, acetylcholinesterase, cell culture, choline acetyltransferase, interleukins.
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P09-18

Involvement of PKR in mediating the activation of caspases in β-amyloid peptide neurotoxicity

K. C. Suen, M. S. Yu, J. Hugon, K. F. So and R. C. C. Chang
Department of Anatomy, Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Hong Kong

β-Amyloid peptide (Aβ) has been considered to play an important role in the pathogenesis of Alzheimer's disease (AD). Aβ has been shown to induce neuronal apoptosis. Cysteine-dependent aspartate-specific proteases (caspases) are a crucial mediator in apoptosis. Among 14 known caspases, caspase-1, -2, -3, -6, -8, -9 and -12 have been found to be involved in Aβ-induced neuronal apoptosis. PKR, a double-stranded RNA-dependent serine/threonine protein kinase has been reported to be significantly involved in apoptosis. Once activated, PKR phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), resulting in inhibition of global protein synthesis. We have recently reported that PKR is activated in Aβ-induced neuronal death. Degenerating neurons in the brains of AD patients also displayed high immunoreactivity of phosphorylated PKR and eIF2α. In addition to the inhibition of global protein synthesis by phosphorylating eIF2α, PKR might have other roles in mediating neuronal apoptosis induced by Aβ. In the present study, we aim to investigate whether PKR can mediate activation of caspases (caspase-2, -3, -8 and -9) in neurons exposed to Aβ. Our results showed that caspase-2, -3, -8 and -9 were less activated in primary cortical neurons from PKR-knockout mice than those from wild-type mice when the neurons were treated with 25 μM of Aβ for 16 h. This suggests that PKR might play a crucial role in regulating the activation of caspases in Aβ-induced apoptosis. We are now further studying how the possible mechanisms underlying PKR-mediated activation of caspases in neuronal apoptosis induced by Aβ.

Keywords: amyloid, apoptosis, caspases, protein kinase, protein phosphorylation.

P09-19

PERK might not be involved in β-amyloid peptide-induced endoplasmic reticulum stress in neurons

K. C. Suen, M. S. Yu, J. Hugon, K. F. So and R. C. C. Chang
Department of Anatomy, Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Hong Kong

β-Amyloid peptide (Aβ) has been proposed to play a significant role in the pathogenesis of Alzheimer's disease. Endoplasmic Reticulum (ER) stress has been suggested to be one of the mechanisms leading to Aβ-induced neuronal cell death as some of the ER stress-related markers including caspase-12, Grp78 and Gadd153 were reported to be involved in Aβ toxicity. PERK, a PKR-like kinase has been suggested to be activated under ER stress. It phosphorylates eukaryotic initiation factor 2 alpha, resulting in inhibition of global protein synthesis. Involvement of PERK has been shown in many ER stress models such as DTT- and thapsigargin-triggered ER stress. However, little is known about its roles in Aβ toxicity in neurons. In the present study, we investigate whether this kinase is activated in neurons exposed to Aβ. Our results showed that Aβ can induce ER stress in cultured cortical neurons in terms of up-regulation of GRP78 and activation of caspase-7 which has recently been proposed to be associated with ER stress. While Aβ can induce ER stress in neurons, western-blotting analysis on PERK showed that amino acid residue at threonine-980 was not phosphorylated in Aβ neurotoxicity. Yet, it is activated by other ER stress inducers such as DTT and thapsigargin. This differential participation of PERK in ER stress in neurons suggests that PERK might not be required in all types of ER stress such as Aβ-induced ER stress. Moreover, there might be some unknown signals which are necessary for the activation of PERK but they are inhibited in Aβ toxicity.

Keywords: amyloid, caspases, protein kinase, protein phosphorylation, stress.

P09-20

Reduction of intracellular calcium increase provides partial protection against β-amyloid peptide neurotoxicity

K. C. Suen, J. Hugon, K. F. So and R. C. C. Chang
Department of Anatomy, Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Hong Kong

β-Amyloid peptide (Aβ) has been proposed to play an important role in the pathogenesis of Alzheimer's disease. One of the proposed mechanisms of Aβ toxicity is the disturbance of intracellular calcium homeostasis. Aβ can induce an increase in intracellular calcium in neurons, probably due to influx from extracellular space or release from intracellular organelles such as endoplasmic reticulum (ER). In this notion, depletion of calcium from the ER can further extend the toxicity of calcium. Prolonged ER calcium depletion could trigger ER stress. In the present study, we focus on whether modulation of intracellular calcium levels or reduction of calcium release from the ER could provide neuroprotection against Aβ. Our results showed that neurons cultured in low-calcium medium were not resistant to Aβ toxicity, suggesting that extracellular calcium might not be the main source of Aβ toxicity. Then, we tried to focus on intracellular calcium release from the ER. Our data demonstrated that three ER calcium release modulators, 2-aminoethoxydiphenyl borate, xestospongin C and FK506 attenuated Aβ toxicity evaluated by the release of LDH, quantification of apoptotic nuclei, PARP cleavage and caspase-3 activation. Yet, an intracellular calcium chelator, BAPTA-AM could not reduce Aβ-induced caspase-3 activation, indicating that simply reduction of intracellular calcium might not be a good therapeutic strategy against Aβ toxicity. Interestingly, our data showed that the three ER calcium modulators reduced up-regulation of GRP78 induced by Aβ, suggesting the significance of ER stress in Aβ toxicity.

Keywords: amyloid, apoptosis, calcium, caspases, stress.

P09-21

Expression and function of dual genes system of tyrosine hydroxylase gene and aromatic L-amino acid decarboxylase gene *in vitro*

H. Yang, Y. Su, C. Duan, C. Zhao, Q. Xu and H. Yang
Beijing Institute for Neuroscience, Beijing Center for Neural Regeneration and Repair, Capital University of Medical Sciences, Beijing, P.R.China

Objective: The characteristic pathological changes of Parkinson's disease include a severe loss of dopamine neurons in the substantia nigra and a severe decrease in dopamine in the striatum. The enzymes of TH and AADC in the biosynthetic pathway for dopamine were considered to be low. A promising approach to the gene therapy of PD is augmentation of enzymes in the biosynthetic pathway for dopamine.
Methods: Human TH and AADC genes were recombinded into retrovirous vectors pLHCX and pLNCX2 respectively. Then pLHCX/TH and pLNCX2/AADC were transfected into packaging cell line PA317 with liposome. The PA317/TH and PA317/AADC were selected by antibiotics. Gene expression was examined by methods of immunohistochemistry and in situ hybridization. The catalytic activity of two-cloned enzyme genes was assessed *in vitro* by HPLC-EC.
Result: Immunohistochemistry staining showed that TH and AADC were expressed efficiently *in vitro*. Both TH and AADC mRNA were transcripted in PA317 cell lines by using *in situ* hybridazation. HPLC-EC experiments revealed that transfected cells produced a significantly higher level of dopamine and L-dopa than untransfected cell, especially added cofactor. The two genetically modified cells can improve L-dopa and dopamine in response to administered substrate.
Conclusion: The present results suggested that not only recombinant TH and AADC genes were successfully expressed *in vitro*, but also the results of enzyme activity assays indicated that they had functional activities respectively. These results have set up a way for *in vivo* gene therapy of Parkinson's disease with TH and AADC genes.
Keywords: aromatic L-amino acid decarboxylase, gene therapy, Parkinson's disease, retrovirous vector, tyrosine hydroxylase.

P09-22

Axonal loss and α-synuclein expression in MOG-induced chronic relapsing EAE in the rat

R. Reynolds and D. Papadopoulos
Department of Neuroinflammation, Division of Neuroscience, Imperial College London, Charing Cross Campus, London, UK

The accumulation of axonal loss is one of the important contributors to progression of disability in Multiple Sclerosis (MS) and is known to begin early in the disease. Here we test the hypothesis that axonal loss determines permanent neurological impairment in inflammatory demyelination. EAE was induced in adult female DA rats by injection of mouse myelin oligodendrocyte glycoprotein (MOG) in IFA. Animals were grouped into those that exhibited a chronic progressive disease course and those that showed a chronic relapsing–remitting course. Demyelination, axonal loss and inflammation were quantified at varying time points. In progressive EAE, axon loss, demyelination and inflammation all correlated significantly with clinical severity scores. In the lateral and dorsal funiculi the decrease in axon number, compared with controls, reached 64.5% by 40 days after the first symptoms. In the late chronic stage of relapsing–remitting EAE only axonal loss correlated with clinical severity scores. Classification of axons into groups according to size showed that small caliber axons were preferentially lost in both relapsing and progressive EAE. These results provide evidence that axonal loss can determine irreversible neurological disability in inflammatory demyelinated lesions of the spinal cord, similar to those seen in MS. Axons in MOG-induced EAE lesions displayed both SMI-32 and APP immunoreactivity, suggesting similar axonal changes to those seen in MS. Interestingly, α-synuclein immunostaining, negligible in control CNS tissue, was greatly upregulated in motoneurons, astrocytes and oligodendrocytes in areas of inflammation. In situ hybridization confirmed that there was an increase in α-synuclein mRNA levels in motoneurons.
Keywords: axon degeneration, demyelination, EAE, inflammation, multiple sclerosis.

P09-23

Nonapoptotic neurodegeneration in the cerebral cortex of young adult wistar rats prenatally exposed to ethanol

F. A. Fakoya and E. A. Caxton-Martins
Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

Apoptosis has been adduced as an important neurodegenerative mechanism following prenatal ethanol exposure (PEE). This study investigated the hypothesis that this effect of PEE persists into adulthood. Dated pregnant female adult wistar rats were divided randomly into groups A and B ($n = 6$) and C ($n = 4$). Group A received a daily ethanol dose of 5.8 g/Kg body weight/day, on the 9th, 10th, 11th, and 12th day of gestation by intragastric intubations, at 16.00 hours (PEE); group B was pair-fed with the ethanol dams and received an isocaloric solution of sucrose to substitute for the ethanol in the experimental group, for the same duration (PF), while group C received standard chow (Ladokun Feeds, Ibadan, Nigeria) (C) and water *ad libitum*. The animals were allowed unto normal parturition. The neocortexes of the adult offsprings were examined at 42 days of age using immunohistochemical (TUNEL staining method for apoptosis) and neurohistological (Nissil, Luxol-Fast-Blue-Nissil and Bodian silver staining) techniques. The microanatomy of the neocortex from the treatment group were distorted, particularly the layer V pyramidal neurons, which consisted mostly of pyknotic pyramidal neurons, with broken apical dendrites, collapsed cell bodies, obliterated nuclei and nucleoli. Intracellular neurofibrillary tangles were not observed. The TUNEL reaction was negative in both groups. Apoptosis does not appear to play a role in the mechanism of action occurring in this age group of animals; these neurodegenerative changes possibly underlie the neurobehavioural deficits that have been variously described.
Keywords: apoptosis, cerebral cortex, neurodegeneration, prenatal ethanol exposure, pyknosis.

P09-24

5-HTTPR is associated with altered neocortical [^{3}H]citalopram binding and anxiety in Alzheimer disease

S. W. Tsang,† C. P. Chen,* P. T. Francis,‡ P. T. Wong,† I. Spence,† M. M. Esiri,§ T. Hope§ and M. K. Lai*
**Department of Clinical Research, Singapore General Hospital, Singapore; †Department of Pharmacology, National University of Singapore, Singapore; ‡Dementia Research Laboratory, King's College London, UK; §Department of Neuropathology & Psychiatry, Oxford University, UK*

Late onset Alzheimer disease (AD) is the most common cause of dementia in the elderly. However, AD is also characterized by neuropsychiatric behaviors like anxiety. The finding that the serotonergic system is affected in AD, and that selective serotonin (5-HT) reuptake inhibitors (SSRIs) are effective in the treatment of anxiety disorders, led us to query whether alterations in 5-HT transporter (5-HTT) function may underlie anxiety in AD. In addition, we examined whether the long variant (L) of a functional polymorphism in the 5-HTT gene promoter region (5-HTTLPR), which results in increased 5-HTT expression and 5-HT reuptake, may be associated with anxiety in AD. Therefore, we measured the affinity and density of 5-HTT sites in the postmortem neocortex by radioligand binding with [^{3}H]citalopram and correlated the binding parameters with 5-HTTLPR genotype and prospectively assessed behavioral data. We found that patients with prominent anxiety had higher densities of [^{3}H]citalopram sites in the temporal cortex, as well as higher frequencies of the LL genotype, compared with never-anxious patients. The odds ratio for the LL vs. the SS or SL genotypes was 6.25 [95% CI (1.14–37.04)]. These results suggest that 5-HTTLPR may be a risk factor for anxiety in AD via its effect on neocortical 5-HTT expression, and provides support for the rationale of using SSRIs for the treatment of behavioral symptoms in AD.
Keywords: Alzheimer's disease, behavior, gene polymorphism, radioligand, serotonin uptake.

P09-25

Anti-inflammatory effect of lovastatin in multiple sclerosis by inducing Th2 immune responses involving GATA3/STAT6 transcription

N. Narender,* G. Shailendra,* S. Avtar† and S. Inderjit*
**Department of Pediatrics, Medical University of South Carolina, Charleston; †Department of Pathology Ralph H. Johnson V.A. Medical Center, Charleston, Charleston, SC, USA*

Statins, HMG-CoA reductase inhibitors, used as lipid lowering drugs have recently been identified as an immunomodulators. We found that *in vivo* treatment of SJL/J mice with lovastatin significantly reduces the duration and clinical severity of active and passive EAE, an animal model of MS. Lovastatin induced expression of GATA3, which is a master controller of IL-4, an important Th2 ?cytokine. Lovastatin specifically inhibited T-bet, a master regulator of IFN- ?B?B signaling pathway by preventing I?in Th1 cells and inhibited the NF-degradation. Further, it inhibited the tyrosine phosphorylation of Jak2, Tyk2, and phosphorylation of STAT4 and up-regulated STAT6 phosphorylation. The inhibition of Jak-STAT4 pathway by lovastatin resulted in decreased T cell proliferation and Th1 differentiation. As result of this, Th1 pro-inflammatory) were inhibited, thereby inducing the Th2 (IL4, IL5,? and TNF?cytokines (IFN and IL10) cytokine responses. The infiltration of CD4 and MHC-II positive cells to the CNS was significantly less in treated mice. These findings strongly B and STAT4?emphasize that lovastatin attenuated EAE by blocking T-bet, NF-signaling in T cells, while it induced the Th2 cell related transcription factors (GATA3 and STAT6), suggesting its use in the treatment of MS and other Th1 cell-mediated inflammatory diseases.

Keywords: cytokines, EAE, signal transduction, statin, T-cells.
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P09-26

Statins induce MAP kinase-dependent activation of brain microglia

X. Bi, M. Baudry, J. Liu, L. Fu, Y. Yao and G. Lynch
University of California Irvine, Irvine, USA

Statins are currently being evaluated as potential treatment to reduce the risk of Alzheimer's disease (AD). As AD is associated with brain inflammation, we determined the effects of prolonged statin treatment on markers of brain inflammation in cultured hippocampal slices. Treatment with mevastatin or simvastatin produced a concentration- and time-dependent activation of microglia throughout the hippocampus. Microglia exhibited both an increase in number as well as a typical morphological change from slim cell body with few ramified processes to round, macrophage-like cells without processes. Furthermore, a subpopulation of activated microglia exhibited increased TNFa expression, in particular in the polymorph layer of the dentate gyrus. Statin-induced microglial changes were prevented by treatment with mevalonate but not with cholesterol, indicating that the effects of statins were due to activation of intracellular cascades not related to cholesterol levels. In addition, these effects were almost completely prevented by treatment with a MEK inhibitor, PD98059. Our results indicate that statin treatment of cultured hippocampal slices induces rapid changes in microglia by interfering with intracellular cascades independent of cholesterol.

Keywords: cholesterol, cytokines, hippocampus, MAP kinase, microglia, statin.

P09-27

Role of PPARγ on amyloid-β generation

M. Sastre, I. Dewachter, E. Rosen, G. Landreth, C. Haass, T. Klockgether, F. van Leuven and M. T. Heneka
Department of Neurology, University of Bonn, Bonn, Germany

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to decrease the risk and delay the onset of AD. The nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ) is directly activated by NSAIDs. To determine whether the effects of NSAIDs on APP processing are mediated by PPARγ activation, we incubated N2a cells transfected APPSW with several pro-inflammatory cytokines together with ibuprofen or PPARγ agonists. Total Aβ was detected by immunoprecipitation and blotting or by ELISA. Immunostimulation by inflammatory cytokines increased amyloid-β (Aβ) generation. When immunostimulated neuroblastoma cells were incubated with NSAIDs, PPARγ agonists or co-transfected with PPARγ cDNA, the amount of Aβ decreased to control levels. These changes were blocked with PPARγ antagonists. To further support our hypothesis, mouse embryonic fibroblasts PPARγ null were used. β-secretase (BACE) activity, expression and transcription was found altered by these treatments. In conclusion, our data indicate an effect of inflammation on APP cleavage, apparently dependent on β-secretase activity and suggest that PPARγ mediate protective effects of NSAIDs in AD.

Keywords: Alzheimer's disease, amyloid, cytokines, inflammation, neuroblastoma.

P09-28

Study of dopaminergic neuron and protein in normal and parkinsonism conditions in common tree shrew brain (tupaia glis)

V. Anupunpisit, T. Tantikraisorn and S. Pakdeeronachit
Anatomy department, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand

Parkinson's disease is a chronic neurodegenerative disorder which is characterized by the loss of dopaminergic neurons of substantia nigra (SN) and decreased striatal dopamine levels. We have investigated the dopaminergic neuron in common tree shrew, which is phylogenetically related to insectivores and primate. Coronal sections through SN and dorsal striatum (caudate and putamen nuclei) were determined for immunoperoxidase localization of the dopamine synthesizing enzyme, tyrosine hydroxylase (TH). The highest densities of TH-labeled neurons were observed in SN area. Interestingly, the pattern of these neurons is organized as clear and separated layers, which is very similar to primate brain. Residual dopamine terminals in striatum showed the lower intensity of TH-labeling. However, the striatum demonstrated patchy characteristic, which is shared by other mammal brains. Moreover, immunoblotting analysis revealed strong TH-immunoreativity in SN but less activity in striatum. The condition of parkinsonism has been introduced, then the alpha synuclein protein produced in SN would be implied as the marker of the neuron loss. The immunoreactivity to anti alpha synuclein has been focused and is now ongoing.Thus, immunological analysis of TH and alpha synuclein might provide the means for characterizing and localizing marker proteins in parkinsonism and other neurological disorders involving dopaminergic system.

Keywords: immunoblotting, immunocytochemistry, neurodegeneration, Parkinson's disease, tyrosine hydroxylase.

P09-29

Inhibition of the expression of tyrosine hydroxylase in alpha-synuclein-transfected dopaminergic cells

X. Zuo,† S. Yu,* M. Zhou,* Y. Li,* Q. Fu,* Y. Huang,* K. Ueda† and P. Chan*
**Department of Neurobiology; †Beijing Institute of Geriatrics, Xuan Wu Hospital, CUMS, China*

The aberrant expression and aggregation of alpha-synuclein (aSyn) has been demonstrated to be toxic to dopaminergic neurons and has been implicated in the pathogenesis of Parkinson's disease. However, little is known about the correlation of the abnormality of aSyn with the expression of tyrosine hydroxylase (TH), a rate-limiting enzyme for the synthesis of dopamine neurotransmitter. In this study, a dopaminergic cell line, MES23.5 was transfected with pcDNA with wild-type aSyn cDNA construct and stable transformants were obtained. Immunocytochemical studies revealed that the number of TH-positive cells were greatly decreased after several passages of aSyn-transfected cells, while that of vector control cells was not affected. Western blot analyses demonstrated that the aSyn-transfected cells expressed much higher aSyn protein when compared with control cells, while the TH levels in aSyn-transfected cells were dramatically reduced. Confocal laser microscopy showed that no apparent abnormal aggregates of aSyn were observed in the aSyn-transfected cells. Although the TH expression in MES23.5 cells was dramatically inhibited by the presence of a large amount of aSyn, the growth and proliferation of these cells were not affected. Apparently, neither necrotic nor apoptotic cell death was observed. These results suggest that an abnormal expression of aSyn may inhibit TH synthesis in dopaminergic cells, which may eventually lead to the degeneration of nigral neurons in diseased brains.

Keywords: alpha-synuclein, Parkinson disease, tyrosine hydroxylase.

P09-30

The effect of amyloid precursor protein 17mer peptide on neurodegeneration in D-galactose induced aging of murine brain

Z. Yongmei, Z. Zhiwei, J. Zhijuan and S. Shuli
Beijing Research Laboratory for Brain Aging, Xuanwu Hospital, Capital University of Medical Sciences, Beijing, China

Objective: To observe the effect of beta-amyloid precursor protein (APP) 17mer peptide on memory and learning ability, hippocampal ultrastructure as well as the expression of neurotrophin-3 (NT-3), nerve growth factor (NGF) in the brain of D-galactose induced aging of murine brain (DGAM). APP17mer peptide is the 319–335 peptide sequence of APP.
Methods: DGAM was produced with D-galactose, and APP 17mer peptide was injected as a therapertic agents. Water maze test, hippocampal ultrastructural study and immunohistochemistry were done 8 weeks.
Results: (1) APP17mer peptide improved the performance of DGAM in water maze test. The whole-length swimming time of DGAM protected with APP17mer peptide was significantly shorter with fewer mistakes than that of DGAM group. (2) Hippocampal neurons of DGAM group showed swelling, ridge fragmentation, dissolution and disappearance of mitochondria, degranulation of rough endoplasmic reticulum. While the hippocampal neurons of DGAM treated with APP17mer peptide did not show marked swelling, mitochondria, rough endoplasmic reticulum had milder damage than that in DGAM group. (3) DGAM group vs. C group showed significantly reduced expression of NT-3 and NGF in hippocampal neurons on immunohistochemistry. Treatment with APP 17mer peptide normalizes the expression of NT-3 and NGF in DGAM.
Conclusion: APP17mer peptide may upgrade the biochemical function of neurons through enhancing the synthesis of NT-3 and NGF in the brain of DGAM. APP17mer peptide improves the neurodegeneration caused by D-galactose.

Keywords: aging, amyloid precursor protein, nerve growth factor, neurodegeneration, neurotrophins.

P09-31

Methionine oxidation and the toxic principle of the β-amyloid peptide from Alzheimer's disease

K. J. Barnham, G. D. Ciccotosto, A. K. Tickler, F. E. Ali, D. G. Smith, N. A. Williamson, Y. H. Lam, D. Carrington, D. Tew, G. Kocak, I. Volitakis, F. Separovic, C. J. Barrow, J. D. Wade, C. L. Masters, R. A. Cherny, C. C. Curtain, A. I. Bush and R. Cappai
Department of Pathology, The University of Melbourne, and The Mental Health Research Institute of Victoria, Australia

The amyloid β-peptide is toxic to neurons and it is believed that this toxicity plays a central role in the progression of Alzheimer's disease. The mechanism of this toxicity is contentious. Here we report that an Aβ peptide with the sulfur atom of Met35 oxidized to a sulfoxide is toxic to neuronal cells and this toxicity is rescued by catalase and the metal chelator clioquinol. Rescuing the toxicity with catalase implicates H_2O_2 in toxicity. Unlike the unoxidized peptide, Met(O)Aβ is unable to penetrate lipid membranes to form ion channel-like structures and β-sheet formation is inhibited, phenomena that are central to some theories for Aβ toxicity. Our results show that, like the unoxidized peptide, Met(O)Aβ will coordinate Cu^{2+} and reduce the oxidation state of the metal, and so still produce H_2O_2.

Keywords: Abeta, Alzheimer's disease, amyloid, oxidation, toxicity.

P09-32

Two monoclonal antibodies reveal distinct subcellular localizations of α-synuclein immunoreactivity in the rat brain

S. Yu, Y. H. Li, M. Zhou, X. M. Liu, X. H. Zuo and P. Chan
Department of Neurobiology, Xuan Wu Hospital, Capital University of Medical Sciences, Beijing, China

α-Synuclein has been implicated in the pathogenesis of Parkinson's disease. However, the function of this protein remains unclear. Although the protein was initially nominated as synuclein for its localizations in both presynaptic terminals and nucleus of the neuron, the nuclear location has not been consistently confirmed by the subsequent studies. In the present study, by using recombinant human α-synuclein to immune Balb/c mice, fusing the spleen cells with the myeloma cells, and screening the hybridoma cells with ELISA, immunohistochemistry and Western blot, we successfully obtained two clones that produced monoclonal antibodies specifically against α-synuclein. Both of the antibodies detected a 18 kD protein in either the rat brain homogenate or the human brain homogenate, which was completely absorbed by pre-incubation of the antibodies with the human recombinant α-synuclein. Both antibodies stained the α-synuclein in the Lewy body and Lewy neurite in the brain of patient with Parkinson's disease. Very interestingly, when the two antibodies were applied to the rat brain immunohistochemically, they revealed distinct localizations of the α-synuclein immunoreactivity, with one antibody detecting the α-synuclein in only the synaptic terminals and another one in both the presynaptic terminals and the nuclei of the neuron. As the two antibodies recognized the different parts of the α-synuclein according to the epitopes demonstrated by phase polypeptide display technique, we suggest that the distinct subcellular localization of α-synuclein in the neuron may imply a conformational change or modification of the protein between the nucleus and the presynaptic terminal.

Keywords: α-synuclein, brain, immunohistochemistry, monoclonal antibody, neuron.

P09-33

Intermolecular interaction between α-synuclein and tyrosine hydroxylase in the regulation of dopamine biosynthesis

Y. H. Li, H. Yu, S. Yu, X. M. Liu, A. Zhang and P. Chan
Department of Neurobiology, Xuan Wu Hospital, Capital University of Medical Sciences, Beijing, China

Objective: To investigate the effects of α-synuclein on dopamine biosynthesis.
Methods: Both recombinant proteins α-synuclein and tyrosine hydroxylase were prepared by *E. coli* expression. The activity of the tyrosine hydroxylase was assayed by measuring the concentration of L-Dopa derived from L-tyrosine. And intermolecular interaction between α-synuclein and tyrosine hydroxylase were analysed by polyacrylamide gel electrophoresis and western blot.
Results: The activity of tyrosine hydroxylase was markedly decreased with presence of α-synuclein. On the contrary, tyrosine hydroxylase appears to interact with α-synuclein and affects its kinetics.
Conclusion: Our data suggest that the activity of tyrosine hydroxylase can be affected by α-synuclein.
Keywords: α-synuclein, biosynthesis, dopamine, intermolecular interaction, tyrosine hydroxylase.

P09-34

The effects of mutations in tau on microtubule assembly

I. Ginzburg, A. Lange, H. Levi and H. Rosenmann
Neurobiology Department, Weizmann Institute of Science, Rehovot, Israel

Tau proteins, are located mainly in neuronal cell body and axons, and function in microtubule (MT) assembly and stabilization. In neurodegenerative disorders including Alzheimer's disease tau proteins aggregate into paired helical consisting of hyperphosphorylated tau,which binds poorly to microtubules. PP2A phosphatase colocalized on microtubules and dephosphorylates tau. We have characterized the double mutant P301S and K257T, associated with severe AD phenotype and add potential sites for phosphorylation. Biochemical and morphological analyses performed in the P19 stable living cell line demonstrate that the double mutant tau protein: (a) does not co-localize with MTs *in vivo*; (b) cannot associate efficiently with MTs and promote their assembly; (c) does not contribute to neurite formation; (d) upon neuronal differentiation, aggregates of tau proteins are observed, which are not colocalizeded with MTs; (e) tau biochemical properties; heat stability and acid solubility are not affected. The ability of wild type and dominant negative forms of PP2A to co-assemble into MTs, adjacent to tau is tested. This research will investigate the interactions between tau and PP2A, and their effect on tau dephosphorylation, MT structure, and assembly in neuronal cells. In addition, these mutations do not show gross changes in the biochemical characteristic of tau, may focus testing pharmacological treatments to prevent or dissolve the pretangle aggregates formed.
Keywords: Alzheimer's disease, axon, microtubule proteins, neurons, tau protein.

P09-35

The oxidative stress effect of 1-methyl-4-phenyl-pyridinium on dopaminergic cell line MES23.5

H. Ding, X. S. Li, Z. X. Hong, X. Y. Ling and C. Biao
Beijing Geriatric Institute, China

The pathological character of Parkinson's Disease (PD) is the loss of dopaminergic neuron in substantia nigra. The etiology of dopaminergic neuronal death in PD remains elusive. The environmental factors are becoming more and more emphasized in recent years. 1-methyl-4-phenyl-pyridinium (MPP+), the reactive metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropypridine (MPTP), one of the environment factors, is the inhibitor of mitochondrial complex I. In our research, we found that MPP+ can reduce the viability of dopaminergic cell line MES23.5. Cellular viability was significantly decreased under the higher concentration (more than 200 mM) in a concentration and time dependent manner. After exposure to MPP+, the OH-, superoxide dismutase (SOD), mitochondrial membrane potential (AΨM), reactive oxidant species (ROS) were changed respectively. Our results suggest that the oxidative stress plays an important role in the toxic effect of MPP+ on dopaminergic cell MES23.5.
Keywords: cell death, dopamine, mitochondria, oxidative stress, Parkinson's disease.

P09-36

Caspase-3 activity is induced by cerebrospinal fluid from multiple sclerosis patients in neuronal cells

C. Cid, J. C. Álvarez-Cermeño, J. Plaza, M. Salinas and A. Alcázar
Serv. Bioquímica-Investigación and Neurología, Hospital Ramón y Cajal, Madrid, Spain

Neuronal apoptosis has recently been implicated in multiple sclerosis (MS), a demyelinating disease of the central nervous system. Cerebrospinal fluid (CSF) from MS patients induces apoptotic cell death of neurons in culture, and apoptotic neurons have been identified in MS lesions and in experimental autoimmune encephalomyelitis (EAE). Caspases are essential for the regulation of apoptosis. Among them, caspase-1 and caspase-3 play a crucial role in the inflammatory process and in the execution step of apoptosis respectively. The activity of these caspases in neurons treated with CSF from MS patients is studied. Besides, we determine whether it is possible to rescue neuronal cells using apoptotic inhibitors. Caspase-like enzyme activity was measured in cell extracts from CSF-treated cultured neurons by a fluorimetric assay based on the cleavage of Ac-YVAD-amc and Ac-DEVD-amc for caspase-1 and caspase-3 activities respectively. Treatment of neuronal cells with MS CSF significantly induced caspase-3 activity, whereas caspase-1 activity was not induced. This caspase-3 activity was inhibited *in vitro* by z-VAD-fmk and Ac-DEVD-cmk. These two caspase inhibitors were used to treat *in vivo* CSF-incubated neuronal cells. Examination of neuronal survival by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and DNA fragmentation detected *in situ* by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) revealed that these caspase inhibitors completely blocked the MS CSF-induced neuronal death. These findings show that neuronal cells were rescued from MS CSF-induced death by caspase inhibitors and support an important role for caspase-3 in apoptotic signaling pathway in MS disease.
Keywords: apoptosis, caspases, cerebrospinal fluid, multiple sclerosis, neurons.

P09-37

Correlation of very long chain fatty acids accumulation and inflammatory disease progression in childhood X-ALD

A. S. Paintlia,* A. G. Gilg,* M. Khan,* A. K. Singh,† E. Barbosa* and I. Singh*

**Department of Pediatrics, Medical University of South Carolina; †Department of Pathology and Laboratory Medicine, Medical University of South Carolina and Ralph H. Johnson V. A. Medical Center, Charleston, SC, USA*

Childhood adrenoleukodystrophy (cALD) is the most frequent clinical phenotype of the defective X-ALD gene, resulting in accumulation of very long chain fatty acids (VLC fatty acids) with subsequent inflammatory demyelination and death in children. The study was carried out to understand the role of inflammatory mediators involved in the neurobiology of cALD by comparing the differential expression of the inflammatory mediators. Histopathological examinations of brain sections indicated extensive demyelination and accumulation of infiltrates in perivascular cuffs in plaque area (PA) and inflammatory area (IA) compared with normal looking area (NLA) of the cALD and controls. The PA had the excessive accumulation of cholesterol ester (25–30-fold), VLC fatty acids (eight to 12-fold) and exhaustive depletion of cholesterol (60–70%) and sphingomyelin (50–55%) in comparison with controls. The mRNA expression of cytokines (IL-1α, IL-2, IL-3, IL-6, TNF-α and GM-CSF), chemokines (CCL2, -4, -7, -11, -16, -21, -22, CXCL1, CX3CL1 and SDF-2) and iNOS in IA was significantly increased compared with NLA of the cALD and controls determined by super gene array, semi-quantitative RT-PCR and immunohistochemistry. These results indicate that accumulation of VLC fatty acids contents in the membrane domains associated with signal transduction pathways may trigger the inflammatory process through activation of resident microglia and these resulting in loss of myelin and oligodendrocytes.

Keywords: human childhood X-ALD brain, inflammatory cytokines and chemokines, super gene array, very long chain fatty acids.

P09-38

Experimental destruction of substantia nigra dopaminergic neurons initiated by lipopolysaccharide

J. Liu, S. Chen and Z. Liu

Department of Neurology, Rui Jin Hospital, Shanghai, Peoples Republic of China

Objective: To study lipopolysaccharide (LPS)-induced injury of substantia nigra dopaminergic neurons and investigate the relationship between immunity and the pathogenesis of Parkinson's disease (PD).

Methods: Lipopolysaccharide was stereostatically injected into left substantia nigra of rats. 14, 21 and 28 days after injection, the circling behavior was observed by intraperitoneal injection of apomorphine, midbrain dopaminergic neurons were identified by using tyrosine hydroxylase (TH) immunohistochemistry, the contents of dopamine and its metabolite in the striatum and substantia nigra were measured by high performance liquid chromatography (HPLC), and the nigral neuronal apoptosis was detected by TUNEL, double-labelling for microglia and inducing-nitricoxide synthase (iNOS) was performed to visualize brain slides.

Results: The circling behavior ipsilateral to LPS injected-side was induced in some rats 21 and 28 days following injection, but not 14 days. Compared with the PBS-treated rats, the marked loss of TH-positive cells in left substantia nigra was identified 21 and 28 days after LPS treatment, but 14 days. The 30–70% reduction of dopamine and its metabolite (DOPAC) of the left striatum and substantia nigra was found at 21 and 28 days following injection, whereas no significant change was observed at 14 days. At the same time, 21 and 28 days after LPS injection, positive double-labelling immunostaining for microglia and iNOS was seen in the injuried substantia nigra, but not 14 days after LPS injection.

Conclusions: LPS intranigral injection could injury the dopaminergic neurons in the substantia nigra. Thus, it suggests that immunity may be involved in the pathogenesis of Parkinson's disease.

Keywords: dopamine, lipopolysaccharide, microglia, Parkinson's disease.

P09-39

Developmentally induced oxidative stress coincides with beta-amyloid plaque deposition in transgenic Tg2576 mice

J. Apelt and R. Schliebs

Paul-Flechsig-Institute for Brain Research, University of Leipzig, Leipzig, Germany

The molecular mechanisms of beta-amyloidogenesis in Alzheimer's disease are still poorly understood. Anti-inflammatory and antioxidant agents have been observed to delay onset or to slow down the progression of Alzheimer's disease. To reveal whether oxidative stress and local inflammatory events may trigger or contribute to beta-amyloid deposition, a transgenic mouse (Tg2576) that express the Swedish double mutation of human amyloid precursor protein and develop Alzheimer-like beta-amyloid deposits at late ages may represent an appropriate approach. In Tg2576 mouse brain, cortical levels of beta-amyloid (1–40) and (1–42) steadily increase with age, but significant deposition of fibrillary beta-amyloid into cortical areas does not occur before postnatal age of 10 months. The aim of the present study was to address the hypothesis whether the age-related occurance of oxidative stress and proinflammatory cytokines coincide with the developmental pattern of beta-amyloid plaque deposition in Tg2576 mouse brain. The activities of superoxide dismutase assayed in cerebral cortical tissue from Tg2576 mice steadily increased between postnatal ages of 9 and 12 months at which age the highest activity during the whole period of life was detected. A similar developmental profile was observed for the activity of glutathion peroxidase. The levels of cortical nitric oxide, reactive nitrogen species (NOx), and interleukin-1beta demonstrated peak values around 9 months of age. The developmental temporal coincidence of increased levels of reactive nitrogen species and antioxidative enzymes with the onset of beta-amyloid plaque deposition strongly suggests the involvement of oxidative stress in triggering beta-amyloidogenesis.

Keywords: Alzheimer's disease, amyloid, nitric oxide, superoxide dismutases, transgenic mouse.

P09-40

Oxidative stress induced intranuclear accumulation of α-synuclein in MES23.5 dopaminergic neurons

S. Xu, M. Zhou, A. Zhang and P. Chan

Beijing Geriatrics Institution, Beijing, China

Parkinson's disease is the most common movement disorder of the elderly. Its pathological characteristics are the loss of dopaminergic neurons in the substantia nigra. So far, the mechanisms underlying neurodegeneration in PD are unknown, but the findings that α-synuclein accumulates in Lewy bodies and the mutations of α-synuclein cause familial PD suggest that a-synuclein participates in the pathophysiology of PD. Some studies have shown that oxidative stress is involved in the neuronal degeneration and can promote the aggregation of α-synuclein in the cytoplasm. We set to further investigate the role of α-synuclein in oxidative stress induced toxicity in dopaminergic neurons. In our study, MES23.5 cell line were treated with H_2O_2 (200 μM) and harvested at 10 min, 30 min, 1 h, 2 h, 4 h and 8 h following the treatment respectively. Fluorescent double labeling was performed using anti-tyrosine hydroxylase (TH) and anti-α-synuclein primary antibodies; Thioflavine S staining was coupled with α-synuclien immunofluorescent staining. The cytoplasmic and nuclear protein were extracted respectively for western blot.

Results: α-Synuclein-like immunoactivity in the cytoplasm was decreased in a time-dependent manner after the cells exposure to H_2O_2. However, accumulation of α-synuclein appeared in the nuclei at H_2O_2 treatment 10 min, and rapidly increased up to 8 h, while the TH expression also reduced in a time-dependent manner. The thioflavine S staining showed that the α-synuclein in nuclei is not aggregated.Our data indicate that the H_2O_2 treatment can induce the intranuclear accumulation of α-synuclein in dopaminergic neurons, but further study is needed to reveal the exact role of α-synuclein in response to oxidative stress in dopaminergic neuron.

Keywords: neurodegeneration, neurons, neurotoxins, oxidative stress, tyrosine hydroxylase.

P09-41

Locus ceruleus cell death and noradrenaline depletion augments learning and memory deficit in APP2576 transgenic mice

M. Ramanathan, L. Dumitrescu-Ozimek, M. Sastre, T. Klockgether and M. T. Heneka
Department of Neurology, University of Bonn, Bonn, Germany

Locus ceruleus (LC) degeneration and subsequent loss of noradrenergic terminals in projection areas are an early event in the course of Alzheimer's disease (AD). Memory deficits in mice overexpressing human amyloid precursor protein APP2576 have been correlated with increased Aβ deposits, and synaptic and cholinergic dysfunction. The consequences of LC degeneration for AD have not yet been clarified. We used APP2576 mice to test whether noradrenergic depletion increases changes of behaviour, memory and neuronal integrity. APP2576 and control mice received a systemic injection of selective neurotoxin DSP4 and after six months of injection, mice were subjected to open field exploration, spatial memory test and brain immunohistochemical evaluation for different markers. The results suggest that APP2576 mice exhibited working and reference memory deficits in radial arm maze compared with wild-type controls. DSP4 aggravated the observed cognitive deficit and produced anxiety in comparison with untreated APP2576 mice. Furthermore neuronal evaluation with neuN, acid fuchsin and Fluro-Jade staining and Aβ immunostaining indicate increased neuronal loss along with Aβ deposits in frontal cortex and hippocampus of APP2576 mice. The ChAT positive neurones in oriens, lacunosum and radiatum areas were found to be significantly reduced with DSP4 treatment. In conclusion, depletion of the noradrenergic system augments Aβ accumulation, neuronal cell death, behavioural alterations and memory deficits in APP2576 transgenics but not in wild-type controls. This result suggests that early LC degeneration is permissive for Aβ-induced neurodegenerative alterations and may therefore contribute to the course of AD.

Keywords: amyloid beta, behaviour, cholineacetyl tranferase, noradrenaline, transgenic mouse.

P09-42

Nitric oxide donors protect against 1-methyl-4-phenyl pyridinium-induced experimental parkinsonism in rats: evidence for antioxida

R. Banerjee, K. S. Saravanan, K. M. Sindhu and K. P. Mohanakumar
Division of Neurosciences, Indian Institute of Chemical Biology, Calcutta, India

Nitric oxide (NO) with free radical properties plays a controversial critical role under a variety of physiologic and pathologic conditions. We investigated the effects of NO donors such as S-nitroso-N-acetylpenicillamine (SNAP), 3-morpholinosydnonimine hydrochloride (SIN-1), sodium nitroprusside (SNP) and nitroglycerin (NG) in nigrostriatal dopaminergic toxicity caused by the potent parkinsonian neurotoxin, 1-methyl-4-phenylpyridinium (MPP+) *in vivo* in rats and in MPP+ induced hydroxyl radical (.OH) generation in mitochondria *in vitro*. NG, SNAP and SIN-1 caused significant attenuation of MPP+ induced .OH generation in mitochondria. Administration of MPP+ by intranigral infusion in rats to produce nigrostriatal damage was significantly attenuated by co-infusion of NG, SNAP and SIN-1 but not by SNP. NG, SNAP and SIN-1 exposed for 48 h to remove NO when administered similarly, failed to protect against MPP+ induced neurotoxicity *in vivo*. Long-term exposed SNP when administered intranigrally, caused dose-dependent depletion of striatal dopamine. These results indicate that selective NO donors can protect dopaminergic neurotoxicity caused by MPP+ by releasing NO in the nigral region and by virtue of its OH scavenging action. This study warrants the neuroprotective role of NO in dopaminergic neurodegeneration.

Keywords: neurotoxins, nitric oxide, Parkinson's disease, striatum, substantia nigra.

P09-43

Aβ sequestration in the periphery as a possible therapeutic approach for Alzheimer's disease

Y. Matsuoka and K. Duff
Center for Dementia Research, Nathan Kline Institute/New York University School of Medicine, Orangeburg, New York, USA

Amyloid beta (Aβ) is one of the pathological features of Alzheimer's disease and the reduction of Aβ is considered a primary therapeutic target. Reduction of Aβ is considered a primary therapeutic target of Alzheimer's disease (AD). One of the proposed therapeutic approaches for AD has been the reduction of brain Aβ by raised (or passively injected) anti-Aβ antibodies. Through vaccination in mice, plasma Aβ was significantly enhanced concomitant with a reduction of brain Aβ, suggesting that the anti-Aβ antibodies may act as a peripheral sink as well as an activator of microglial phagocytosis. Here, we have investigated whether agents with high Aβ binding affinity might be effective in altering the periphery/brain dynamics leading to a reduction of brain Aβ. We treated two amyloid-forming transgenic mice with agents, gelsolin and GM1, that have high affinity for Aβ, but which are unrelated to an immune modulation, for 2–3 weeks by intraperitoneal injections. After drug treatment, the level of brain Aβ was significantly reduced, and in some treatment protocols, plasma Aβ was significantly enhanced. However, direct brain infusion with an Aβ binding agent did not reduce insoluble brain Aβ, indicating that Aβ binding agents can reduce brain Aβ without entering the brain. Overall, we found that Aβ binding agents could significantly reduce AD-type brain amyloidosis without entering the brain. We propose that in general, compounds that sequester plasma Aβ in the periphery could reduce or prevent brain amyloidosis, thus suggesting new therapeutic or prophylactic options that may be more flexible, more reliable and less likely to cause side-effects than immunization based therapies.

Keywords: Alzheimer's disease, amyloid, transgenic mouse.

P09-44

Inactivation of glycogen synthase kinase by estrogen receptor and its implications to Alzheimer's disease pathogenesis

S. Goodenough, C. Pietrzik, D. Manthey, E. Koo and C. Behl
Institute of Physiological Chemistry and Pathobiochemistry, Johannes Gutenberg University, Mainz, Germany

The sex steroid hormone estrogen exerts a diverse array of biological functions both during development and in the adult, also including nonreproductive organs such as the brain. Moreover, estrogen has been proposed to mediate neuroprotection and to act as a preventive compound in neurodegenerative disorders such as Alzheimer's Disease (AD). Here we examine estrogen's role in key pathological processes associated with AD. We found that in neuronal cells estrogen induced an increased phosphorylation at ser-9, and therefore inhibition, of glycogen synthase kinase 3β (GSK), a modulator of nerve cell survival and a kinase involved in the hyperphosphorylation of tau. This inhibition is estrogen receptor (ER) dependent and is mediated via the activation of the MAPKinase pathway. Further, ERα coimmunoprecipitates with GSK, representing one potential mechanism for GSK inhibition. The inactivation of GSK also occurred *in vivo*, particularly in the hippocampus, a prime target in neurodegeneration. The implications of these findings were then examined with respect to AD-associated pathological processes. First, GSK may regulate the estrogen-driven increase in sAPPα release from neuronal cells. The inhibition of GSK with lithium chloride increased sAPPα and decreased total Aβ release in APP overexpressing cells. Secondly, estrogen decreased the GSK-mediated hyperphosphorylation of tau *in vivo*. Taken together, we have found a novel functional and molecular link between estrogen and GSK. In addition, we have established a molecular link between estrogen and GSK to central process of the pathogenesis of AD.

Keywords: Alzhiemer's disease, amyloid, estradiol, tau protein.

P09-45

Cholesterol oxidase activity mediates neurotoxicity of the Alzheimer β-amyloid cuproprotein

A. I. Bush, L. Puglielli, S. Nagano and D. M. Kovacs
Massachusetts General Hospital, Charlestown, MA, USA

Beta-amyloid (Aβ) accumulation is causally related to the pathogenesis of Alzheimer's disease, however the mechanism of neuronal demise is unclear. Both cholesterol metabolism and brain metal dyshomeostasis are implicated in AD pathogenesis. We have previously characterized Aβ as a metalloprotein that possesses redox active, high-affinity Cu^{2+} binding sites. Oligomeric complexes of Cu^{2+}/Aβ catalyze the generation of hydrogen peroxide using O_2 and biological reducing agents as substrates. Cu^{2+}/Aβ insert themselves in to lipid membranes, and so we characterized the ability of these complexes to utilize cholesterol as a substrate for hydrogen peroxide formation. We found that Cu^{2+}/Aβ converts cholesterol into a well-known pro-apoptotic neurotoxin, cholestene-3β-one, in a catalytic manner (Km = 5.2 μM, *V*max = 46 nM/min) that is inhibited by chelators, such as clioquinol. This activity is accompanied by hydrogen peroxide production and is greatest for Aβ 42 > Aβ 40 ≫ rat Aβ, paralleling the involvement of these peptides in neuropathology. In cell culture, the toxicity of Cu^{2+}/Aβ correlates with the conversion of cellular cholesterol to cholestene-3β-one. Inhibition of this rogue activity may contribute to the potential therapeutic effectiveness of both clioquinol (a chelator) and statins.

Keywords: Alzheimer's disease, amyloid, cholesterol, copper, oxidative stress.

P09-46

Dopamine dynamic changes and behavioural assessment in advanced Parkinson's disease and levodopa induced dyskinesia models

W. M. Zhu, D. I. Finkelstein, K. D. Buxton, J. Drago and M. K. Horne
Department of Neurology, Monash University, Clayton, Australia

In previous studies we showed that following partial lesions of the Substantia nigra (SNpc), surviving dopaminergic (DA) neurones sprout and reinnervate the striatum. We have also shown that the terminals newly formed by sprouting have impaired function of the dopamine transporter (DAT). In Parkinson's Disease (PD) there is progressive loss of SNpc DA neurones and chronic levodopa treatment almost always results in dyskinesia. We have hypothesized that in PD compensatory sprouting and the associated terminal dysfunction may be responsible for both dyskinesia and also motor fluctuations. This study examined this hypothesis. Rats and marmosets received partial lesions of the SNpc and were left until compensatory sprouting would have occurred. Voltammetry was used to measure DA reuptake from synaptosomes and *in vivo* and DA release. Animals received L-dopa, which induced dyskinesia in the marmosets. Both *in vivo* and *in vitro* studies showed that DA uptake was functionally impaired following SNpc damage, but that the D2 receptor was functional in that application of a D2R agonist causing increased DA uptake. DA release induced by medial forebrain bundle (MFB) stimulation was not decreased. The release and uptake of DA in the reinnervated striatum was measured and this was compared with normal and following a dose of L-dopa. In marmosets, the response will be correlated with the degree of dyskinesia. These results indicate that the decrease in dopamine uptake of regenerated terminals combined with the increased terminal arbour could be the basis for both dyskinesia of Parkinson's Disease and tardive dyskinesia.

Keywords: L-dopa, dopamine, neurotransmitter, Parkinson's disease, striatum.

P09-47

Specific degeneration of beta-amyloid-associated cholinergic structures in transgenic APPsw mice

A. O. Ihunwo, H.-J. Luth, J. Apelt, T. Arendt and R. Schliebs
Mbarara University Medical School, Mbarara, Uganda

Cholinergic dysfunction is a consistent feature of Alzheimer's disease and the interrelationship between beta-amyloid deposits, inflammation and early cholinergic cell loss is still not fully understood. To characterize the mechanisms by which beta-amyloid and proinflammatory cytokines may exert specific degenerating actions on cholinergic cells ultrastructural investigations by electron microscopy were performed in brain sections from transgenic Tg2576 mice that express the Swedish double mutation of the human amyloid precursor protein and progressively develop beta-amyloid plaques during aging. Both light and electron microscopical investigations of the cerebral cortex of 19-month-old transgenic mice revealed a number of pathological tissue responses in close proximity of beta-amyloid plaques, such as activated microglia, astroglia proliferation, increased number of fibrous astrocytes, brain edema, degeneration of nerve cells, dendrites and axon terminals. Ultrastructural detection of choline acetyltransferase (ChAT)-immunostaining in cerebral cortical sections of transgenic mice clearly demonstrated degeneration of ChAT-immunoreactive fibers in the environment of beta-amyloid plaques and activated glial cells suggesting a role of beta-amyloid and/or inflammation in specific degeneration of cholinergic synaptic structures.

Keywords: Alzheimer's disease, choline acetyltransferase, glia, inflammation, ultrastructure.

P09-48

Staurosporine prevents inhibitory effect of amyloid β1-42 on hemicholinium-3 sensitive choline transport in NG108-15 cells

V. Dolezal, J. Novakova, V. Lisa and S. Tucek
Institute of Physiology CAS, Prague, Czech Republic

Cholinergic cell line NG108-15 (1) enhances its neuronal and cholinergic phenotype during differentiation induced by simultaneous presence of cAMP and dexamethasone in growth medium. Amyloid β1–42 (Aβ) present in growth medium in submicromolar concentrations during differentiation prevents the expression of functional N-type calcium channel. Now we have investigated an influence of Aβ on a cholinergic marker, hemicholinium-3 (HC-3) sensitive choline uptake, in control and differentiated cells. Four-day-lasting differentiation resulted in an increase of both HC-3 binding and HC-3 sensitive choline uptake. Submicromolar concentrations of Aβ present in growth medium during differentiation reduced the increase of HC-3 binding in a concentration-dependent manner and at a concentration of 100 nM also choline uptake. In contrast, Aβ present only during measurement at a concentration of 1 μM was not effective. In differentiated cells, staurosporine (100 nM) increased whereas tetradecanoylphorbol acetate (TPA; 1 μM) decreased choline uptake when present 5 min prior to and during measurement. In Aβ treated cells staurosporine also increased choline uptake while TPA lost its inhibitory effect. Both staurosporine and TPA when included in growth medium during differentiation together with Aβ reversed its inhibitory effect. These observations indicate that protein kinase C regulates choline uptake and that the inhibitory effect of Aβ at submicromolar concentrations may be mediated by a persistent increase of protein kinase C activity.

Keywords: amyloid, choline uptake, cholinergic neuron, differentiation, protein kinase.

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P09-49

Gene expression profile changes in the caudate of PD patients

P. Chan, P. B. Chan and Y. Cai
Beijing institute of Geriatric, Beijing, China

Objective: To understand the gene expression profiles in the caudate of PD patients.
Methods: Genes differentially expressed in caudates of PD patients were detected using affymatrix-chips which provided an analysis of 7070 genes in total. In comparison with matched normal control, 170 genes have shown an altered expression pattern, including 47 turned up, and 123 turned down. These genes are associated with multiple physiological functions, such as oxidative stress, transcription regulation, signal transduction pathway, and neurotransmission etc.
Conclusion: Multiple physiological processes as well as genes are thought to be associated with Parkinson disease. The importance of maintaining both the release of neurotransmitters and regulative interplay among different neurons was also discussed.
Keywords: chip, dopamine, Parkinson's disease.

P09-50

Characterization of α-synuclein in human lymphocytes

S. H. Kim,* C. J. Heo,* P. S. Im,* T. B. Ahn,† B. S. Jeon† and Y.-H. Suh*
**Department of Pharmacology, College of Medicine, National Creative Research Initiative Center for Alzheimer's dementia and Neuroscience Research Institute, MRC, Seoul National University, Seoul; †Department of Neurology, Seoul National University, College of Medicine, Clinical Research Institute and MRC, Seoul National University Hospital, Seoul, Republic of Korea*

Human α-synuclein is a 140 amino acid protein with little or no secondary structure. α-Synuclein is expressed at high levels in the brain and enriched in neural synaptic terminals but its physiological function remains largely unknown. More recently, α-synuclein has been shown to be one of the principal components of Lewy bodies, neuronal inclusions that are found in diverse human neurodegenrerative disorder including the Lewy body variant of Alzeheimer's disease, diffuse Lewy body disease, Parkinson's disease, multiple system atrophy. Therefore, α-synuclein and its abnormal protein aggregation have been thought to play a role in the pathogenesis of these neurodegenerative diseases known as α-synucleinopathies. In order to understand their etiology and pathogenesis, it is crucial to identify the normal function of α-synuclein. It was previously reported that the splicing ?variant of α-synuclein is expressed in heart, skeletal muscle, and pancreas. In our study, we identified the expression in spleen and confirmed the expression of α-synuclein in isolated human PBMC. The physiological role in lymphocytes showed relationship with apoptosis, induction of caspase-8, and caspase-9 with inflammatory cytokines. This result suggest that the accumulated α-synuclein might be involved in regulation of cell viability by the interaction with proteins such as synphilin-1.
Keywords: apoptosis, caspase, lymphocyte, neurodegeneration, Parkinson's disease.

P09-51

Development of mtDNA-transfused cell models of Alzheimer's disease and application in Chinese herb study

L. Zhang, L. Li and F. Liu
Department of Pharmacology, Xuan-wu hospital of CUMS, Beijing, P.R. China

Objective: To develop mtDNA-transfused cell models (cybrids) of Alzheimer's disease (AD), observe the pathological characteristics of cybrids, and investigate the pharmacological effects of Chinese herb component SSY-p3 on the cybrid models.
Methods: The ρ^0 cells without mitochondria were fused with the platelets from AD patients, aged and young donors. Cytochrome oxidase (COX) activity was determined by microplate assay. The specific fragment of mtDNA was detected by PCR and gel electrophoresis. Reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and cytosolic calcium were determined by laser scanning confocal microscopy and flow cytometry.
Results: (1) The successful development of cybrids was verified by the evidence that cybrids grew and proliferated well in selective medium without uridine, recovered COX activity and had the mtDNA-specific fragment. (2) COX activity in AD cybrids decreased compared with aged and young control ($p < 0.05$). (3) ROS in AD cybrids was higher than that in aged and young control ($p < 0.05$). Incubation of Chinese herb component SSY-P3 (100 μg/mL) with AD cybrids for 24 h declined their ROS production. (4) MMP of AD cybrids was lower than that in aged and young control ($p < 0.01$). SSY-P3 Incubation elevated their MMP by 80.4% ($p < 0.05$). (5) In comparison of young control, the basal cytosolic calcium of AD cybrid was increased, and the regulatory ability to calcium was decreased. SSY-P3 decreased basal cytosolic calcium and enhanced regulatory ability to calcium in AD cybrids.
Conclusion: mtDNA transfused cells (cybrids) of AD can be used as a good model to investigate the pathogenesis related to mitochondria and to screen the drugs. Chinese herb component SSY-P3 may be beneficial to retarding or treating Alzheimer's diseases.
Keywords: Alzheimer's disease, calcium, free radicals, membrane potential, mitochondria.

P09-52

The parkinsonian neurotoxin, 1-methyl-4-phenyl pyridinium (MPP+), affects voltage activated calcium channels in DRG neurons

G. Chandra,* D. Büsselberg† and K. P. Mohanakumar*
**Division of Neurosciences, Indian Institute of Chemical Biology, Calcutta, India; †Physiologie, Universitätsklinikum Essen, Essen, Germany*

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes parkinsonian syndromes in human and animals, which is featured by hypoactivity of dopamine (DA) neurotransmitter system resulting from degeneration of dopaminergic neurons of substantia nigra *pars compacta* (SN*pc*) region of the brain. NMDA receptor-mediated cell death, altered intracellular Ca^{2+} homeostasis, and functional run-down of ligand-gated ($GABA_A$) channels of dopaminergic cells are some propositions that point to an active involvement of membrane depolarization as an initial event in MPTP-neurotoxicity. In the present study, we reported the effects of MPTP and and its active neurotoxic metabolite, 1-methyl-4-phenyl pyridinium ion (MPP^+) on voltage-activated inward currents of cultured dorsal root ganglia (DRG) neurons derived from neonatal rats. Voltage-activated Ca^{2+}, K^+ and Na^+ currents were elicited in the whole-cell configuration of the patch clamp technique, and were isolated using specific internal solutions. Externally applied MPTP and MPP^+ showed opposing effects on the electrical properties of DRG neurons and only MPP^+ showed significant inhibitory effects. Voltage-activated Ca^{2+} currents were dose- and time-dependently inhibited by MPP^+, while higher doses of MPP^+ evoked an inhibitory effect on the voltage-gated K^+ currents. The voltage-activated Na^+ currents remained unaltered. The present study indicates that, MPP^+ reduces currents through voltage-gated Ca^{2+} channels, and this may have direct bearing on the neurotoxicity caused by this potent neurotoxin.
Keywords: calcium channel, dorsal root ganglion cells, methylphenyl tetrahydropyridine (MPTP), Parkinson's disease, patchclamp.

P09-53

Effect of acetylcholinesterase inhibitors in streptozotocin-induced Alzheimer's disease in rats

M. Hina and K. P. Mohanakumar
Division of Neurosciences, Indian Institute of Chemical Biology, Calcutta, India

Therapy of Alzheimer's disease (AD) by acetylcholinesterase inhibitors has brought in a host of problems because of its severe side effects including tremor. Though anticholinesterases cause an increase in the level of acetylcholine in the brain, other classes of neurotransmitters may also be affected. To understand the extent of involvement of biogenic amine neurotransmitters in the actions of anticholinestarase in AD, we investigated the effects of physostigmine and tacrine in an animal model of the disease. Rats were administered intracerebroventricularly with streptozotocin (STZ), and treated with physostigmine or tacrine. Discrete brain regions such as nucleus caudate putamen (NCP) and hippocampus were dissected and analyzed for the biogenic amines employing HPLC-electrochemistry. Cognitive functions were assessed using a Plus-maze apparatus. Cholinesterase activity increased significantly in striatum where as reduced in HP on 21st day following STZ administration. While the cholinesterase inhibitor caused severe inhibition of acetylcholinesterase in the striatum, heightened serotonin levels were observed in the striatum and hippocampus. Initially after the STZ injection, DA and 5-HT levels are reduced significantly on day 14, in NCP and HP. DA level was increased significantly in both NCP and HP along with 5-HT in NCP alone, on 21day following icv injection of STZ. Norepinephrine was unaffected. Treatment with cholinesterase inhibitor in the STZ pretreated animals, compensates the STZ-induced changes in DA and 5-HT in NCP as well as HP. The behavioral and biochemical observations from the present study indicate the neurochemical basis of the side effects (eg. tremor, hallucination, etc.) observed following anticholinesterase therapy in AD.

Keywords: acetylcholinesterase, Alzheimer's disease, hippocampus, streptozotocin, striatum.

P09-54

C-terminal cytoplasmic fragments of APP induce neurotoxicities through the transcriptional modulated mechanism

K.-A. Chang, J.-W. Ha, H.-S. Kim and Y.-H. Suh
Department of Pharmacology, Nat'l Creative Research Initiative Centre for AD and Neuroscience Research Institute, MRC, College of Medicine, Seoul Nat'l University, Seoul, Korea

Amyloid β peptide (Aβ) is a metabolic product of the amyloid precursor protein (APP) that accumulates in neuritic plaques in Alzheimer's disease (AD). However, although it is believed that Aβ 40 and Aβ 42 form is pathogenic, intramembrane cleavage of APP generates both Aβ and C-terminal cytoplasmic fragment. An earlier report suggests that Aβ may not be the sole active component involved in the pathogenesis of AD. Therefore, other cleavage products of APP may contribute to the neurodegenerative process and their potentialities need to be explored. There are recent reports that a shorter 50 amino acid C-terminal fragment (CTF50) is generated from a second transmembrane cleavage site in APP as well as CTF99, CTF59(CTF57) and CTF31. However, it is unclear whether CTF50 as well as CTF99 and how CTF50 or CTF99 could contribute to neurodegeneration. In the present study, we have found that the expression of CTF50 and CTF99 lead to apoptosis by caspase-3 activation in neuronal cells unrelated with nucleus signaling. In addition, expression of CTF50 and CTF99 in neuronal cells induce the up-regulation of GSK-3β gene in the nucleus through interaction with Fe65 and CP2/LSF/LBP1 transcription factor, whereas the deletion of YENPTY domain does not. This occurred with an increase in the active form of GSK-3β, accompanied by the induction of tau phosphorylation leading to apoptosis. From these results, we suggest tnat CTF50 and CTF99 might contribute to the neuronal degeneration in AD through not only mitochondrial dysfunction but also the nucleus signaling involved in transcription of genes including GSK-3β.

Keywords: Alzheimer's disease, C-terminal fragment of amyloid precursor protein, Fe65, glycogen synthase kinase 3β.

P09-55

No abstract received/abstract withdrawn

P09-56

A novel protein interacts with α-synuclein

Q. L. Ma,* B. Chen,* and K. Uéda*,†
**Department of Neurology, Xuanwu Hospital of Capital University of Medical Sciences, Beijing, China; †Department of Neurochemistry, Tokyo Institute of Psychiatry, Tokyo, Japan*

Objectives: α-synuclein, a pre-synaptic molecule, was originally identified in Alzheimer's disease amyloid and named non-Ab component precursor (NACP). Later, two missense mutations in the α-synuclein gene had been identified in autosomal dominant familial Parkinson's disease (PD). Increasing evidence indicated that α-synuclein was involved in the pathogenesis of several neurodegenerative disorders, including Parkinson's disease (PD), Lewy body disease (LBD) and multiple system atrophy (MSA). However the mechanisms underlying α-synuclein involvement are unclear. To investigate the potential factors that may act as protein-interaction partners of α-synuclein, we carried out studies to identify proteins that interact with α-synuclein.
Methods: Affinity column chromatography was used to isolate the novel proteins that may interact with α-synuclein from human brain tissues. The interaction was confirmed by immunohistochemistry, immunoblotting and immunocytochemistry of cultured neurons.
Results: A novel protein that interacts with α-synuclein was identified.
Conclusion: Our results appear to suggest that a novel protein that may interact with α-synuclein was identified and it may play an important role in the pathogenesis of neurodegenerative disorders.

Keywords: α-synuclein, neurodegenerative disorders.

P09-57

Effect of medicinal herbs on acetylcholinesterase inhibition and tau phosphorylation

M. T. W. Ho, D. C. C. Wan and P. C. Shaw
The Chinese University of Hong Kong, Hong Kong, Hong Kong

We have examined three extracts from Chinese medicinal materials for inhibitory activity against acetylcholinesterase (AChE). AChE activity was determined by the colorimetric Ellman assay. Both the aqueous and ethanol extracts were tested for AChE inhihitory activity. The aqueous extract (10 mg/mL) of *Radix Paeoniae Rubra* (Chi Shao) showed modest 55% inhibition against human AChE while that of *Radix Paeoniae Lactiflorae* (Bai Shao) gave 35% inhibition at the same concentration. The ethanol extract of Chi Shao showed mild 30% inhibition at 10 mg/mL, while Bai Shao gave a 70% inhibition with ethanol extract at the same concentration. Chi Shao and Bai Shao belong to the family of Ranunculaceae and sometimes they are used interchangeably. Decoctions of Bai Shao can inhibit inflammation. The extracts of *Pericarpium Citri Reticulatae* (Chen Pi) were also examined and both gave a 20% increase in the AChE activity at 50 mg/mL. The extract was found to increase tau protein phosphorylation as detected by tau phospho-dependent antibodies. It supports the possible linkage between AChE activity and tau phosphorylation level. Effect of Chi Shao and Bai Shao on tau phosphorylation will also be discussed.

Keywords: acetylcholinesterase, Alzheimer's disease, antibodies, phosphorylation, tau protein.

P09-58

Involvement of calcium in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced experimental parkinsonism

S. Samantaray and K. P. Mohanakumar
Division of Neurosciences, Indian Institute of Chemical Biology, Calcutta, India

Parkinson's disease, a progressive neurodegenerative disorder, involves the selective degeneration of A_9 substantia nigra neurons in the brain. In the present study, we hypothesize that the active form of the parkinsonian toxin, 1-methyl-4-phenyl pyridinium ion (MPP^+) acts as a Ca^{2+} channel blocker. The study explores the Ca^{2+}-involvement in this selective neurodegeneration at three levels: (i) subcellular level aiming to assess the intrasynaptosomal Ca^{2+} flux using Fura-2AM; (ii) tissue level employing HPLC-electrochemistry to monitor dopamine release from striatal slices; and (iii) organism level involving *in vivo* studies by pharmacologically intervening with L-type dihydropyridine Ca^{2+} channel agonist, (±)-Bay K8644 and antagonist, nicardipine. The active metabolite, MPP^+ was used for *in vitro* and *ex vivo* experiments whereas for *in vivo* investigations, Balb/c mice were injected with MPTP. *In vivo* effects of calcium channel agonist, Bay K8644 and antagonist, nicardipine with and without MPTP were monitored for different parameters such as monoamine oxidase-B activity, tyrosine hydroxylation, MPP^+ formation in the striatum, levels of reduced (GSH) and oxidized glutathione (GSSG), and striatal DA employing HPLC-electrochemistry. In the present study we observed MPP^+ causes an increase in intrasynaptosomal Ca^{2+}, culminating in DA release from the striatum, which is sensitive to L-type dihydropyridine Ca^{2+}, channel modulation. Our studies with (±)-Bay K8644 and nicardipine affirmed the involvement of L-type Ca^{2+} channels in MPTP-neurotoxicity. Relevant findings from the study proved our hypothesis: MPP^+ being a Ca^{2+} channel blocker, acting at the L-type Ca^{2+} channels.

Keywords: Parkinson's disease, methylphenyl tetrahydropyridine, calcium, calcium channel, neurodegeneration.

P09-59

Serotonin 5-HT_{2A} receptor alterations in the postmortem neocortex of behaviorally assessed Alzheimer patients

M. K. Lai,* S. W. Tsang,† M. M. Esiri,‡ J. Keene,‡ T. Hope,‡ P. T. Francis,§ P. T. Wong† and C. P. Chen¶
**Department of Clinical Research, Singapore General Hospital; †Department of Pharmacology, National University of Singapore, Singapore; ‡Departments of Neuropathology, Psychiatry and Ethox, IOHS, Oxford University; §Dementia Research Lab, King's College London, UK; ¶National Neuroscience Institute, Singapore*

Levels of serotonin 5-HT_{2A} receptors were measured by [^{3}H]ketanserin binding in the postmortem frontal and temporal cortex of Alzheimer Disease (AD) patients prospectively assessed for cognition and neuropsychiatric behaviors, as well as matched controls. In agreement with previous studies, 5-HT_{2A} receptor densities were reduced in both regions in AD. However, these receptor alterations were correlated only with dementia severity, and not with premortem behaviors such as depression, psychosis, anxiety and aggression. This study suggests that 5-HT_{2A} levels may be a marker of neurodegeneration in AD but may not be directly involved in neuropsychiatric behaviors, in contrast to results obtained from previous retrospective studies.

Keywords: alzheimer disease, neocortex, neuropsychiatric behavior, serotonin receptors.

P09-60

L-deprenyl attenuates the rotenone-induced dopaminergic neurotoxicity: experimental evidences in rats

K. S. Saravanan, K. M. Sindhu and K. P. Mohanakumar
Division of Neurosciences, Indian Institute of Chemical Biology, Calcutta, India

Parkinson disease (PD) is progressive neurological disorder because of massive degeneration of nigrostriatal dopaminergic neurons. The pathogenesis of PD is unknown, but considerable evidence suggests multifactorial factors including genetic, mitochondrial dysfunction, oxidative stress, excitotoxicity, calcium cytotoxicity, environmental factors and apoptosis. We investigated the role of oxidative damage produced by intranigral infusion of a potent mitochondrial complex-I inhibitor, rotenone and studied the neuroprotective effects with a well-known antiparkinsonian drug L-deprenyl in rats. Unilateral stereotaxic intranigral infusion of rotenone 6 μg caused significant decrease in dopamine levels. L-deprenyl (10 mg/kg) treatment significantly attenuated the DA depletion caused by rotenone. Parallely, a significant decrease in the concentration of GSH was also observed in the SN was reverted by L-deprenyl treatment. L-deprenyl significantly attenuated the rotenone-induced decrease in tyrosine hydroxylase immunoreactivity in striatum. The results suggest that L-deprenyl can rescue the dopaminergic neurons from the rotenone mediated neurodegeneration in this experimental animal model.

Keywords: complex I, deprenyl, glutathione, oxidative stress, Parkinson's disease.

P09-61

Co-transplantation with GDNF and VMC: a better approach in restoration of neurobehavioral function in 6-OHDA lesioned rat model of Parkinson's disease

R. K. Chaturvedi, A. K. Agrawal, K. Seth, Y. Shukla, S. Shukla and P. K. Seth

Developmental Toxicology Division, Industrial Toxicology Research Centre, M. G. Marg, Lucknow, India

During neural transplantation, degeneration of transplanted VMC is very high due to lack of trophic support and mismatch conditions. In the present study an attempt has been made to validate the role of Glial cell line-derived Neurotrophic Factor (GDNF) co-transplanted with fetal VMC in functional restoration in rat model of Parkinson's disease. A significant restoration was observed in amphetamine induced rotation in rats co- transplanted with GDNF and VMC (66%, $p < 0.001$) as compare to VMC alone (42%). Apomorphine induced locomotor activity was restored by 67% ($p < 0.01$), 38% in co-transplanted and VMC alone transplanted rats respectively. Level of dopamine and 3,4 dihydroxy-phenyl acetic acid (DOPAC) in the striatum were significantly restored by 67 and 62% ($p < 0.01$), 42 and 33% ($p < 0.05$) in co-transplanted and VMC alone transplanted rats respectively. A significant restoration was observed in striatum dopamine receptors by 69% in rats co-transplanted with VMC & GDNF, and 45% in those transplanted with VMC alone. The functional viability of transplanted VMC was confirmed by tyrosine hydroxylase (TH) expression and quantification of TH positive cells by image analysis revealed a significant restoration in number of TH-positive cells as well as area of TH expression in co-transplanted animals over VMC transplanted animals. However restoration was more pronounced in the case of microtransplantation approach as compared to macrotransplantation.

Keywords: amphetamine, apomrphine, GDNF, microtransplantation, Parkinson's disease.

P09-62

Supplemental role of antioxidants in fetal ventral mesencephalic cell (VMC) and olfactory ensheathing cell transplantation (OEC)

S. Shukla, K. Agrawal, K. Seth, R. Chaturvedi and P. K. Seth

Developmental Toxicology Division, Industrial Toxicology Research Centre, M. G. Marg, Lucknow, India

Fetal neural transplantation is one of the promising therapy in PD, but major constraint is low survival of transplanted cells (2–5%) first week post-transplantation, less availability of fetal tissue and ethical issues raised due to use of fetuses. Major factors causing low survival are apoptosis, free redical mediated toxicity. In order to overcome free redical mediated toxicity and to enhance the survival of transplanted cells, in the present study an attempt has been made to co-transplant fetal VMC with OEC and antioxidants (10 mM of glutathione and ascorbic acid) to minimize the use of fetal tissue and to protect from oxidative stress respectively. The functional recovery was evaluated at 4 week post transplantation by evaluating selected neurobehavioral, neurochemical and immunohistochemical parameters.THE 6-OHDA lesioned animals exhibited an increase in amphetamine induced locomotor activity (65%) and amphetamine induced circling behaviour (72%) and dopamine receptors (65%).The rats receiving only OEC showed an average 23% recovery, while the rats receiving only VMC showed an average of 42% recovery in these parameters. The rats administered OEC+VMC showed 65% recovery. The antioxidant supplementation to VMC + OEC further enhanced the recovery to 71%.The results suggest that supplementation of antioxidants has beneficial and additive role.

Keywords: Antioxidants, apomorphine, olfactory ensheathing cells, tyrosine hydroxylase.

P09-63

Development of cell models over-expressed with human amyloid precursor protein for the study of amyloid processing and cell death

M. H. Leung,* K. W. K. Tsim† and D. C. C. Wan*

**Department of Biochemistry, The Chinese University of Hong Kong; †Department of Biology, The Hong Kong University of Science and Technology, SAR China*

Human amyloid precursor protein (APP) expression, processing and localization were studied *in vitro* in cell cultures. The aim was to evaluate the impact of over-expression and accumulation of amyloid peptides on cell death. Human amyloid precursor protein isoforms APP695 and APP770 were cloned by RT-PCR. The full coding APP cDNAs were subcloned into mammalian expression vector pCMV-tag1. The pCMV-tag1-APP695 was transfected to a human neuroblastoma cell line, SH-SY5Y and a non-neuronal cell line, HEK293. The cell line stably transfected with APP695 was generated by treatment of the transfected cells with G418 for at least 4 weeks. The accumulation of the insoluble 39-42 a.a. Aβ and the C-terminal fragments formed by aberrant APP processing, particularly by β-secretases was analyzed by Western blots and ELISA. The transfected cells were also tested for evidence of apoptosis and neuronal cell death. Other than secretases, recent data suggest that a brain-specific trypsin-like enzyme neurosin (zyme) might play a role in the processing/degradation of APP. This enzyme was also cloned and characterized to determine its role in APP processing using the established cell models.

Keywords: Alzheimer's disease, amyloid, apoptosis, caspase, immunoblotting.

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P09-64

Attenuation of stimulated calcium responses of PC12 cells by amyloid β peptide

P.-C. Wu,* H.-J. Tsay,* L.-S. Kao,† D.-M. Yang‡ and Y.-J. Sung§

**Institute of Neuroscience. †Faculty of Life Sciences, National Yang-Ming University, Taipei, Taiwan, ROC; ‡Department of Medical Research and Education, Taipei Veterans General Hospital; §Institute of Anatomy and Cell Biology, National Yang-Ming University, Taipei, Taiwan, ROC*

Amyloid peptide (Aβ) forms senile plaques in the brain of patients with Alzheimer's disease (AD). Aβ has been shown to be neurotoxic to cells, but the exact mechanisms are unknown. One possible mechanism of Aβ toxicity involves a calcium dysregulation accompanied with enhanced vulnerability to excitotoxic stimuli. This study examined the effects of Aβ on the calcium signaling using PC12 cell as a model system. Both high potassium (K) and ATP stimulations rapidly elevated cytosolic calcium and followed by a gradual decrease to the resting level in control PC12 cells. Prolonged exposure to Aβ decreased the amplitude of the high K- or ATP-induced cytosolic calcium increase and the high K-induced mitochondrial calcium increase. However, Aβ did not affect the basal calcium level in the cytosol and mitochondria. Aβ treatment had no effect on the calcium storage capacity of? mitochondria and ER, estimated by carbonyl cyanide m-clorophenylhydrazone (CCCP)- and thapsigargin (TG)-induced calcium spike. In the presence of CCCP or TG, similar attenuation of high K-induced elevation of cytosolic calcium was observed. The results indicate that the attenuation of high K- or ATP-induced calcium responses of PC12 cells by prolonged exposure to Aβ is because of a direct effect on the channels or receptor in the plasma membrane rather than on the mitochondria or ER.

Keywords: amyloid peptide, carbonyl cyanide m-clorophenylhydrazone, PC12, thapsigargin.

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P09-65

NMDA receptor dysfunction in Alzheimer's disease

M. R. Hynd, H. L. Scott and P. R. Dodd
Department of Biochemistry, University of Queensland, Brisbane, Australia

The inherent neurotoxic potential of the endogenous excitatory amino acid glutamate, may be causally related to the pathogenesis of AD neurodegeneration. Neuronal excitotoxicity is conceivably mediated by the N-methyl-D-aspartate-(NMDA)-Ca2+-ionotropic receptor. NMDA receptors exist as multimeric complexes comprising proteins from two families – NR1 and NR2(A-D). The polyamines, spermine and spermidine bind to, and modulate NMDA receptor efficacy via interaction with exon 5, an alternatively-spliced, 21 amino acid, N-terminal 'cassette'. AD-associated cognitive impairment may therefore occur via subunit-specific NMDA receptor dysfunction effecting regional selectivity of neuronal degradation. Total RNA was prepared from pathologically spared and susceptible regions from AD cases and matched controls. Quantitation was performed using standard curve methodology in which a known amount of a synthetic ribonucleic acid competitor deletion construct was co-amplified against total RNA. Expression profile analysis of two NR1 mRNA subsets has revealed significant differences in NR11XX mRNA levels in cingulate gyrus, $p < 0.01$; superior temporal cortex, $p < 0.01$ and hippocampus, $p = 0.05$. Differential age-dependent expression for NMDA isoforms was found in pathologically affected regions in AD. Proportionate NR11XX expression in AD cingulate gyrus and temporal cortex was lower at younger ages in AD cases and increased with age. Quantitation of individual NR1 splice-variants revealed a nonselective reduction of all variants containing exon 5. mRNA levels for NR2A and NR2B were similarly reduced in AD susceptible areas, $p < 0.05$. Variations in endogenous NR1 and NR2 mRNA levels suggest a relationship between the NMDA receptor, mediation of excitotoxic neuronal degeneration and AD pathogenesis.

Keywords: Alzheimer's disease, excitatory amino acid receptors, mRNA, neurodegeneration, NMDA.

P09-66

Time-dependent increase in parkin expression during unfolded protein stress in cultured astrocytes

M. C. Lau,* H. L. Choi,* P. H. Jensen† and A. Stadlin*
**Department of Anatomy, Chinese University of Hong Kong, Hong Kong; †Department of Medical Biochemistry, University of Aarhus, Aarhus, Denmark*

Dysfunction of the E3 ubiquitin ligase parkin because of mutations is the cause autosomal recessive juvenile parkinsonism. Parkin protects cells against the toxicity of unfolded proteins and unfolded protein stress has been postulated to be one of the mechanisms underlying nigral degeneration in Parkinson's disease. It was shown recently that unfolded protein stress (UPS) elicited a selective increase in astrocytic parkin expression not shown in neurons in the hippocampus. The current study used 10 μg/ml tunicamycin to induce unfolded protein stress in astrocytes cultured from rat hippocampus, cortex, striatum and mesencephalon to further explore the role of astrocytes upon UPS. At 3, 6, 12 and 24 h after tunicamycin treatment, cells were extracted and the protein expressions of parkin, α-synuclein and BiP (ER) were examined by western blot analysis. Results showed that at 12 and 24 h after treatment, there is an increased expression of ER protein BiP when compared with controls. Concomitantly, there is an increased expression of parkin protein. There is no change in the level of α-synuclein expression at all time points studied. This phenomenon is ubiquitous to astrocytes from all regions studied. This study demonstrates that increased parkin expression is part of a global astrocytic response to unfolded protein stress and indicates that parkin dysfunction may play a wider in neurodegenerative disorders.

Keywords: astrocytes, cell culture, Parkinson's disease, ubiquitin.

P09-67

C-terminal fragments of amyloid precursor protein exert neurotoxicity by transcription dependent mechanism

J.-W. Ha, N. Kim, H.-S. Kim, K.-A. Chang and Y.-H. Suh
National Creative Research Initiative Centre For Alzheimer's Dementia, Department of Pharmacology, College of Medicine, Seoul National University, Seoul, Korea

Amyloid precursor protein (APP) is cleaved in its transmembrane region by γ-secretase. β- and γ-cleavage of APP generates the extracellular amyloid β-peptide (A?), the main component of Alzheimer's disease (AD) senile plaques, and releases a free carboxyl terminal intracellular fragment (APP-CT). APP-CTF (C-terminal fragment of amyloid precursor protein) is recently suggested to form a complex with Fe65 and the histone acetyltransferase Tip60 and may affect gene transcription. In this study, we investigated the effect of C-terminal fragment of amyloid precursor protein on histone acetyltransferase and cytotoxicity. The cytoplasmic tail of APP binds to Fe65 and histone acetyltransferase to increase acetylated histone and also increases cytotoxicity. Moreover, the levels of acetylated histone and cytotoxicity are significantly increased by the histone deacetylase inhibitor sodium butyrate. Dexamethasone, a possible candidate for histone acetyltransferase inhibitor, shows no inhibitory effect on the activity of histone acetyltransferase and cytotoxicity.

Keywords: Alzheimer's disease, cytotoxicity, dementia, neurotoxins.

P09-68

Minocycline exerts protective effects on the neurotoxocity induced by Ab and various C-terminal fragments of APP

Y. Choi, E.-M. Kim, C. H. Park, H.-S. Kim, K.-Y. Shin and Y.-H. Suh
Department of Pharmacology, College of Medicine, National Creative Research Initiative Center for Alzheimer's Dementia and Neuroscience Research Institute, MRC, Seoul National University, Seoul, South Korea

Alzheimer's disease (AD) is a neurodegenerative disorder neuropathologically characterized by the presence of neuritic plaques containing amyloid fibrils and neurofibrillary tangles whose main component is paired helical filament composed of hyperphosphorylated tau. There are numerous lines of evidence that some of the neurotoxicity associated with AD is because of proteolytic fragments of amyloid precursor protein (APP) including amyloid beta peptide (Ab), which is the main component of neuritic plaques. Minocycline is a second-generation tetracycline that effectively crosses the blood–brain barrier. It has remarkable neuroprotective qualities in models of cerebral ischaemia, traumatic brain injury, and Huntington's and Parkinson's disease. However, there is no evidence about neuroprotective effects of minocycline on AD. In this study, we demonstrate that minocycline exerts protective effects on the neurotoxicity induced by Ab and various C-terminal fragments of APP through inhibition of cytochrome c release and caspase-3 activation. And also, we have investigated the effects of minocycline on the learning and memory impairment and reduction of ACh levels and PDH activities in cerebral cortex and hippocampus induced by injection of C-terminal fragments of APP in rats.

Keywords: Alzheimer's disease, amyloid, minocyclin.

P09-69

α-Synuclein induced-apoptosis in human peripheral blood mononular cells

C. J. Heo, S.-H. Kim, I.-P. Sun and Y.-H. Suh
National Creative Research Initiative Centre for Alzheimer's Dementia, Seoul National University, Seoul, South Korea

Human α-synuclein is a 140 amino acid protein with little or no secondary structure. The α-synuclein is expressed at high levels in the brain and enriched in neural synaptic terminals but its physiological function remains largely unknown. More recently, α-synuclein has been shown to be one of the principal componets of Lewy bodies, neuronal inclusions that are found in diverse human neurodegenrerative disorder including the Lewy body variant of Alzeheimer's disease, diffuse Lewy body disease, Parkinson's disease, multiple system atrophy. Therefore, α-synuclein and its abnormal protein aggregation have been thought to play a role in the pathogenesis of these neurodegenerative diseases known as α-synucleinopathies. In order to understand their etiology and pathogenesis, it is crucial to identify the normal function of α-synuclein. It was previously reported that the splicing variant of α-synuclein is expressed in heart, skeletal muscle, and pancreas. In our study, we identified the expression in spleen and confirmed the expression of a-synuclein in isolated human PBMC. The physiological role in lymphocytes showed induction of apoptosis with Caspase-8 and Caspase-9 activation. And also they showed enhanced expression of inflammatory cytokines, like TNF-a and IL-6. This result suggest that the accumulated α-synuclein might be involved in regulation of cell viability by the interaction with proteins such as PKC, ERK, or BAD.

Keywords: apoptosis, caspase, lymphocyte, neurodegeneration, Parkinson's disease.

P09-70

Neurotoxicity and nuclear locolization of APLP2-C-terminal fragments

Y.-J. Xu, K.-A. Chang, H.-S. Kim, E.-M. Kim, C.-H. Park, E.-A. Yuh, Y.-H. Suh
Seoul National University, Medicine, Pharmacology, National Creative Research Initiative Center for Alzheimer's Dementia, Seoul, Korea

β-Amyloid Precursor Like Protein-1 and -2 (APLP1 & APLP2) are processed by the r-secretase and can generate carboxyterminal fragments, such as CT57-59. CT50 and CT31 which generated by ?-secretase and caspase-3, respectively. All processing is similar to what has been reported for AID (APP intracellular Domain). In the present study, we investigated the neurotoxicity and nuclear localization of APLP2-CTFs. Transfection of PC12 cells, rat primary cultured cortical neurons and HEK293cells with APLP2-CT57, CT50, CT31 can induce apoptotic cell death after 48 h; and increase the release of cytochrome c and activate caspase-3 in HEK293 cells. All three CTFs translocate to the nucleus, forming ternary complex with Fe65 & CP2/LSF/LBP1 in the nucleus, and then increase the GSK-3β mRNA and protein levels, inducting of tau phospholation, leading to apoptosis. These results indicate that APLP2-CTFs might contribute to the neuronal death in AD.

Keywords: APLP2-CTFs, Fe65, GSK-3beta, neurotoxicity, nuclear locolization.

P09-71

Effects of an 'enriched environment' on the Alzheimer disease tg2576 transgenic mice

P. E. Vivas-Mejía, J. Felix and S. Peña de Ortiz
University of Puerto Rico, Rio Piedras Campus, Puerto Rico

Recently it has been hypothesized that the level of education and social stimuli that promote positive behavioral and mental activities can delay the onset of Alzheimer Disease (AD). Although, there is not yet a perfect transgenic mouse that shows all of the neuropathological features AD, the use of the AD-transgenic mice has been useful not only to understand many of the molecular and structural bases of AD but also to understand the learning and memory processes. APP695 SWE mice expressing transgenic human APP with the two point 'Swedish' mutations show age-dependent impairments and many pathological features typical of AD. We used the Tg2576 mice (Tg) to test the hypothesis that an Enrichment Environmental can delay the onset of the pathological features of AD in these transgenic animals. Twelve weeks old, 26 animals (14 wild-type littermates and 12 Tg) were used initially in this study. Eight wild-type littermates and six wild-type littermates constitute the enrich group (EG) and eight wild-type littermates and six Tg were kept in standard cages and constitute the control (CG). All groups of animals are subjected to behavioral tests before and after several periods of stimulation in Enriched Environments. So far we have found that at 12 weeks of age and prior to stimulation Tg mice are not different than wild types in place recognition memory task. On the contrary, Tg seems to be impaired in object recognition memory compared with wild types. Ongoing studies will examine the effect of stimulation on hippocampus-dependent and independent behavioral tasks. In addition, we will investigate the underlying differences in pathology and expression of molecular markers between the experimental groups.

Keywords: Alzheimer disease, enrichment environmental, Tg2576.
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P09-72

Novel cognitive improving and neuroprotective activities of *Polygala tenuifolia* willdenow extract, BT-11

S.-M. Ahn, C. H. Park, S. H. Choi, J.-W. Koo, J.-H. Seo, H.-S. Kim, Y.-J. Lee and Y.-H. Suh
Department of Pharmacology, College of Medicine, National Creative Research Initiative Center for Alzheimer's Dementia and Neuroscience Research Institute, MRC, Seoul National University, Seoul, Korea

We performed this study to search a new active constituent that had cognitive enhancing activity and low side effects from natural source. We found that the extract of dried root of *Polygala tenuifolia* Willdenow (BT-11, 10 mg/kg, i.p.) could significantly reverse scopolamine-induced cognitive impairments in rat, using a passive avoidance and a water maze test. We also investigated the effects of BT-11 on the neurotoxicities induced by glutamate (Glu) and toxic metabolites of amyloid precursor protein (APP) such as amyloid protein (A and C-terminal fragment of APP (CT) in primary cultured neurons of rat. The pretreatment of BT-11 (0.5, 3, 5 g/mL) significantly reduced cell death induced by Glu (1 mM), A (10), and CT105 (10 M) in a dose-dependent manner. In addition, BT-11 inhibited acetylcholinesterase (AChE) activity in a dose-dependent and noncompetitive manner (IC50 value; 263.7 g/mL). Our novel findings suggest the possibility that this extract may have some protective effects on neuronal death and cognitive impairments in Alzheimer's disease (AD) or other neurodegenerative diseases central cholinergic dysfunction related to excitotoxicity.

Keywords: AD, APP, BT-11, CT, neurodegeneration.

P09-73

The role of α-synuclein in the BCL2 family expression and PI3-AKT kinase pathway

J.-H. Seo, J.-C. Rah, S. H. Choi, J. K. Shin, H.-S. Kim, C. H. Park, S. Kim and Y.-H. Suh

Department of Pharmacology, National Creative Research Initiative Center for Alzheimer's Dementia and Neuroscience Research Institute, MRC, Seoul National University, Seoul, South Korea

α-Synuclein (SN) is a ubiquitous protein that is highly abundant in the brain and is implicated to play a central role in the pathogenesis of many neurodegenerative diseases including Alzheimer's and Parkinson's disease. Nevertheless this protein is so crucial in the function of the brain and in human brain diseases, the physiological functions and the molecular role of SN in the pathogenesis of neurodegeneration are still unknown. There are many controversies and doubts in its normal physiological functions in the nervous system and its precise roles in the cell death. Here, we report that SN has neuron specific dual neuroprotective and neurotoxic effects depending on its concentration. In the nanomolar levels, SN protected neurons against serum-deprivation, oxidative stress and excitotoxicity through the PI3-Akt signaling pathway. In contrast, both low micromolar of SN and overexpressed SN induced cytotoxicity, which might be mediated by the decreased bcl-2 expression and increased bax expression subsequently followed by cytochrome c release and caspase activation, and by microglia-mediated inflammatory response via the NF-kB and MAPK pathways.

Keywords: Alzheimer's disease, cell death, neurodegeneration, Parkinson's disease, signal transduction.